Iron & Iron Salts Used In Foods 3/12/75

P 35

I R O N
A N D
I R O N S A L T S
U S E D I N F O O D S

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INFORMATICS INC.
6000 Executive Boulevard
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IRON AND IRON SALTS USED IN FOODS

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Summary

Iron and iron salts have been used as medicine for centuries. The ancient Greeks, Egyptians and Hindus prescribed iron as a cure for general weakness - now recognized as a symptom of anemia, and recommended it for both diarrhea and constipation. In the 18th century iron was shown to be a constituent of blood, and by mid-19th century iron was the most popular of therapeutic agents. In the late 1800's, however, numerous studies purported to show that inorganic iron was not absorbed, or hardly so, by the body, and its use fell into disfavor. Not until the early decades of this century did iron therapy regain prestige and renewed application, particularly in large doses. The early use of pure, powdered iron, with little or no toxicity, and an apparent effectiveness to recommend it; for some unknown reason gave way to development, and use of, a wide variety of iron compounds, many with undesirable side-effects. In the instance of the popular ferrous sulfate, numerous grave illnesses and fatalities due to children ingesting the iron tablets intended for an anemic parent were reported. The potential dangers of iron salts as the cause of accidental poisoning have not received the attention from either physicians or the public they deserve. Small children confuse the often brightly colored, sugar-coated iron pills with candies, and many have died from ingesting them.

Several excellent reviews of the literature and history of iron therapy are included in this monograph (146, 357, 321, 61, 6). Of particular interest is a paper by Shanas (308), reviewing the history of powdered iron and recommending a return to elemental iron (reduced) in anemia therapy.

It should be made clear at this point that the normal use of iron and iron salts as dietary supplements in foodstuffs such as flour used in bakery products; and in therapeutic treatment of iron-deficiency anemia is not a cause for concern. Iron is an essential mineral in our diet for the production of hemoglobin, and the minimum daily requirement varies from 6 to 18 mg depending upon age and sex (243). Toxicity of iron compounds is related to very large amounts consumed accidentally, or in a few instances, used in homicides.

Dietary deficiencies in iron and the subsequent necessity for iron supplementation is a serious medical problem (100) but is not the chief interest of this monograph. The reference just noted is a recent study (1970) of the biological availability of various iron compounds from common dietary sources, particularly those that are, or might be, used for food fortification. Ferrous sulfate is used as a reference standard, and anemic young chicks and rats were fed diets with various iron salts added. The relative biological values found are, in order from most to least effective: ferric ammonium citrate, ferrous sulfate, ferrous chloride, ferrous gluconate, ferric sulfate, ferric chloride, ferric pyrophosphate, reduced iron, ferric orthophosphate, sodium iron pyrophosphate, ferric oxide and ferrous carbonate.

An earlier, similar study by Blumberg (33) using anemic rats showed ferric chloride equal in biological activity to ferrous sulfate, which was in turn 4 to 5 times as available as ferric orthophosphate. The report stresses the desirability of using highly assimilable forms of iron in flour and bread enrichment.

Iron retention and hemoglobin regeneration in anemic rats, fed various iron compounds or bread enriched with iron salts, as a measure of the relative effectiveness of the compounds shows the following sequence: ferric chloride is more effective than sodium ferric orthophosphate, is equal to ferric phosphate, is more effective than ferrous iron, and is more effective than sodium iron pyrophosphate (98).

Ferric chloride added as a supplement in feeding experiments with rats has shown a detrimental influence on calcium and phosphorous metabolism (280). It appears that the addition of iron to the diet reduces the amount of available phosphorous and has resulted in rickets in rats, guinea pigs and rabbits. Calcium levels are also decreased.

Ammerman et al. (10) reported on the utilization of iron salts fed to calves and sheep. Radioactive Fe^{59} fed as sulfate, carbonate and chloride was deposited in tissues of the animals in the order listed. Other studies showed the Fe^{59} in Fe_2O_3 significantly less available. Iron-depleted calves fed Fe^{59} as the chloride, $FeCl_3$, showed a 3-5-fold greater tissue deposition than non-depleted calves given the same treatment.

The interrelationship between calcium and iron metabolism has been investigated both from the viewpoint of the influence of calcium on iron absorption and hematopoiesis and from the interference of bone formation by high iron intake. Moore et al. (232) fed rats diets of raw beef, and diets supplemented by $CaCO_3$ and $FeCO_3$. Histological examinations of the livers of the rats and chemical estimations of iron, indicated Fe deposits directly related to Fe intake and irreversibly related to Ca intake.

Preferential use of ferrous carbonate in anemia therapy is suggested by the low degree of toxicity and its effectiveness in raising the hemoglobin level in anemic piglets (69). In dogs, $FeCO_3$ has been shown to be 100 times as safe as ferrous sulfate, 30 times as safe as ferrous gluconate and 15 times as safe as ferrous succinate (68). Some investigators, however, find the carbonate ineffective in the treatment of iron-deficiency anemia in human patients, but suitable for treatment of symptomatic chloranemias (223).

Some attention has been given to the effectiveness of ferrous chloride and the tartrate relative to the kind of preparation given to female patients in a Stockholm hospital. The absorbability of ferrous chloride should be good due to its solubility in water and presumed solubility in lipids; it has been demonstrated effective in anemia therapy. The chloride does, however, possess a disagreeable metallic taste and tends to produce dyspepsia when ingested in large doses. The usual form of administration is as sugar-coated

tablets or in a syrup. In this study, the chloride produced a higher serum iron concentration than the tartrate (249).

Chicks used in anemia studies showed ferric oxide as ineffective; ferrous sulfate and Cu-free ferric chloride stimulated hemoglobin synthesis. Purified ferric chloride was ineffective until minute amounts of copper were added; copper acts as a supplement to iron in hemoglobin synthesis in chicks and rats (84).

Ferric ammonium citrate and other iron compounds were given orally to adult male rats to test hypotheses explaining how iron is absorbed and excreted. Synthesis of C^{14}-labelled ferritin iron in the rat's intestinal mucosa was increased by the citrate three-fold over controls, 4-5 hours after ingestion. Ferritin protein remained unchanged. It is suggested that iron incorporated into ferritin in the intestinal mucosa provides a means for excreting unneeded iron, which then provokes synthesis of apoferritin which traps incoming iron and is then excreted as ferritin (317).

A single instance of ferrous gluconate poisoning in an infant girl is reported. The child ingested 30 or more tablets (0.3 gm). A new Fe chelating agent, desferrioxamine, is credited with assisting the child to a successful recovery (134).

The LD_{50} doses of ferrous gluconate and ferrous sulfate were determined for mice, rats and dogs by oral, intravenous, intraperitoneal and intragastric administration. Subacute, emetic effects were observed over a period of a month, and gastrointestinal distress noted (357).

Ferric pyrophosphate was fed to young anemic dogs in amounts ranging from 200-1000 gamma of Fe/kg body weight/day. A minimal dose of 600 gamma of Fe was determined to be optimal for hemoglobin synthesis. Wheat bran and spinach in amounts supplying 600 gamma of Fe/day replacing the pyrophosphate showed the bran iron almost completely available; the iron in spinach was only 20-40% available (294).

Daily oral administration of 5 mg of ferric pyrophosphate to human infants during their first year showed statistically significant increases in hemoglobin and hematocrit levels up to about nine months of age; at age one year, however, this salutary effect lost significance (89).

With exception of the single ferrous gluconate poisoning case previously noted, ferrous sulfate has been the iron compound accidentally ingested by children, causing acute illness and about 50% fatalities. The sulfate, one of the most commonly prescribed forms of iron for iron-deficiency anemia, has proven fatal in doses of as few as a dozen or so tablets, ingested by infants. Fatal doses of the sulfate have ranged from 40-1600 mg/kg, with an average dose estimated at about 900 mg/kg (145).

Typical cases of human poisoning from ferrous sulfate have been reported in numerous papers with frequent clinical descriptions and treatments (95, 293, 310, 321, 111, 365, 319, 323, 341, 61, 166, 31, 97, 57, 311, 6, 319, 39, 86, 49, 96).

The shock syndrome observed in cases of acute ferrous sulfate poisoning has been attributed to ferritin production in the body (317). Other mechanisms are proposed to explain death in acute poisoning by the sulfate; rabbits have been injected with ferrous sulfate solution, autopsied and examined for gross morphological changes. The gastric mucosa necrosis and hyperanemia following ingestion of large iron doses may produce a breakdown of normal apoferritin-ferritin control mechanism, flooding the plasma with iron and mobilizing alpha and beta globulin acting to protect the ferric iron complex. The uncombined iron acts directly as a vasodepressant, precipitating vascular collapse (42). Histochemical studies in rabbits have suggested that massive iron overload is capable of causing alterations in several cellular oxidative enzymes, including some in the Krebs cycle. Citric and lactic acidemia characteristic of acute ferrous sulfate poisoning could result from damage to Krebs cycle enzymes demonstrated histochemically (367). This author also reports that studies of rabbit necrotic liver cells, following large injected doses of ferrous sulfate, under electron microscopy shows considerable mitochondrial injury, which also suggests a basis for the toxicity of acute iron overload.

Potentiation of iron sulfate absorption from aqueous solutions, tablets and plastic matrices by the addition of ascorbic acid suggests that the combination would be an improvement in anemia therapy over the present use of ferrous sulfate without ascorbic acid (210).

The use of British anti-lewisite (BAL) in therapy of a case of severe ferrous sulfate poisoning with a favorable outcome suggests its use in future, similar cases (311).

Teratogenicity of salicylates in Wistar rats, evidenced by abnormal embryos, lacking otoliths, in the animals fed sodium salicylate is increased by supplementing the salicylate diet with 2 mg of ferrous gluconate; resorptions and malformations are strikingly increased (173).

A study of the side-effects of iron compounds used in iron-deficiency therapy produced results showing nearly half as many patients receiving placebos reporting side-effects as those receiving ferrous sulfate tablets (222 mg Fe) daily. The toleration of oral iron in dosages usually prescribed is low in some patients, who complain of abdominal swelling, constipation, loose stools and nausea (122).

IRON AMMONIUM CITRATE

Chemical Information

I. Nomenclature

A. Common Names

1. Ammonium Ferric Citrate
2. Ferric Ammonium Citrate
3. Iron Ammonium Citrate
4. Soluble Ferric Citrate (the brown form)

B. Chemical Names

1. Ammonium Ferric Citrate
2. Ferric Ammonium Citrate

C. Trade Names

None

D. Chemical Abstracts Services Unique Registry Number

001332985

II. Empirical Formula

Structure undetermined

III. Structural Formula

(Compounds of NH_3, iron and citric acid)

IV. Molecular Weight

Undetermined

V. Specifications

Contains about 9% NH_3, 16.5-18.5% Fe, and about 65% hydrated citric acid. The NAS/NRC questionnaire provides the following assays:

Ferric ammonium citrate, brown form	
Assay (as Fe)	16.5-18.5%
pH (4.5 gm/100 ml H_2O)	5.5-7.0
Ferrous iron	Negative to test
Oxalate	Negative to test
Sulfate	1000 ppm maximum
Lead	10 ppm maximum
Mercury	3 ppm maximum
Arsenic	3 ppm maximum
Ferric ammonium citrate, green form	
Assay (as Fe)	14.5-16.0%
pH (4.5 gm/100 ml H_2O)	3.3-4.3
Limits of impurities as above for brown form	

VI. Description

A. General Characteristics

Reddish-brown granules, garnet-red transparent scales, or brownish-yellow powder. Odorless or slight NH_3 odor; saline, ferruginous taste. The green form, composed of about 7.5% NH_3, 14.5-16% Fe, and 75% hydrated citric acid, appears as transparent scales, pearls, granules or powder; odorless with mild ferruginous taste.

B. Physical Properties

Both forms are highly deliquescent and light-sensitive; very soluble in H_2O and insoluble in alcohol. The green form is readily reduced to ferrous salt by exposure to light.

C. Stability

Ferric ammonium citrate should be kept well-closed and protected from light.

VII. Analytical Methods

Three methods of analysis of brown ferric ammonium citrate in syrup form are described and compared by Salazar and Otalora (300).

1. Complexometric method with EDTA (Ethylenediaminetetraacetic acid sodium salt). EDTA in 0.1 M solution is used to titrate, with sulphosalycylic acid as indicator, and concentrated HCl. The authors regard this method as imprecise, probably because the Fe is in chelate form and acidulation with HCl does not split the complex completely.

2. Method of U. S. Pharmacopeia XII. The authors modified this iodometric method by destroying organic matter with 35% H_2O_2 in alkaline medium after hydrolysis with concentrated HCl. This method yields low values for Fe.

3. British Pharmacopeia Method. This method is given in detail (see report) and is recommended by the authors (300), as modified.

VIII. Occurrence

Ferric ammonium citrate is produced by reaction of black iron oxide (Fe_3O_4) with purified aqueous solution of citric acid and aqueous ammonia. It is not a true compound and the yield is either the brown form or green form depending upon the relative quantities of reactants used.

IRON CARBONATE

Chemical Information

I. Nomenclature

A. Common Names

1. Iron Carbonate
2. Ferrous Carbonate
3. Siderite

B. Chemical Names

1. Ferrous Carbonate

C. Trade Names

Fecarb, Blaud's mass, Vallet's mass

D. Chemical Abstracts Services Unique Registry Number

000563713

II. Empirical Formula

$FeCO_3$

III. Structural Formula

Not applicable

IV. Molecular Weight

115.86

V. Specifications

None (C.P. as food supplement)

VI. Description

A. General Characteristics

Ferrous carbonate occurs in the anhydrous, $FeCO_3$ form, consisting of gray crystals, and a hydrate, $FeCO_3 \cdot H_2O$ which is amorphous.

B. Physical Properties

$FeCO_3$ is insoluble in water: $FeCO_3 \cdot H_2O$ only slightly soluble in water. Both are soluble in acids and aqueous CO_2, and decompose when heated.

C. Stability

Ferrous carbonate should be kept in tightly closed containers

VII. Analytical Methods (Food Chemicals Codex)

General qualitative tests for carbonates and ferrous salts may be used.

VIII. Occurrence

$FeCO_3$ occurs in nature as the mineral siderite. For medical purposes a mixture of 36-41% $FeCO_3$ and honey and sugar is known as Blaud's mass, Fecarb, or Vallet's mass.

IRON CHLORIDE

Chemical Information

I. Nomenclature

A. Common Names

1. Iron Chloride
2. Molysite
3. Lawrencite

B. Chemical Names

1. Ferric Chloride (Molysite)
2. Ferrous Chloride (Lawrencite)

C. Trade Names

None

D. Chemical Abstracts Services Unique Registry Number

007 705 080

II. Empirical Formula

$FeCl_3$ (Ferric)
$FeCl_2$ (Ferrous)

III. Structural Formula

Not applicable

IV. Molecular Weight

$FeCl_3$ - 162.22
$FeCl_2$ - 126.76

V. Specifications

None (C.P. as food supplement)

VI. Description

A. General Characteristics

$FeCl_3$ occurs as hexagonal, dark leaflets or plates. It is red by transmitted light, green by reflected light; sometimes appears brownish-black. The hexahydrate form, $FeCl_3 \cdot 6H_2O$ is brownish-yellow or orange crystalline lumps.

$FeCl_2$ occurs as white rhombohedral crystals, sometimes with green tint. Hydrated forms occur: a dihydrate, $FeCl_2 \cdot 2H_2O$ consists of white monoclinic crystals with pale green tint; a tetrahydrate, $FeCl_2 \cdot 4H_2O$ is pale green to blue-green monoclinic crystals or crystalline powder.

B. Physical Properties

$FeCl_3$ is very hygroscopic; melts and volatilizes at about 300°; bp about 316°; very soluble in water, alcohol, ether, acetone; slightly soluble in CS_2; practically insoluble in ethyl acetate. The usual commercial form is the hexahydrate, which has a slight odor of HCl, is very hygroscopic and the mp is about 37°. It is readily soluble in water, alcohol, acetone, and ether.

$FeCl_2$ is very hygroscopic, mp 674°, bp 1023°. It is freely soluble in water, alcohol and acetone; slightly soluble in benzene; practically insoluble in ether.

C. Stability

Both ferric and ferrous forms are hygroscopic and unstable and should be kept well closed.

VII. Analytical Methods (Food Chemicals Codex)

General qualitative tests for chlorides and ferric and ferrous salts may be used.

VIII. Occurrence

$FeCl_3$ occurs in nature as the mineral molysite.
$FeCl_2$ occurs in nature as the mineral lawrencite.

IRON GLUCONATE

Chemical Information

I. Nomenclature

A. Common Names

1. Iron Gluconate
2. Ferrous Gluconate

B. Chemical Names

1. Ferrous Gluconate

C. Trade Names

1. Fergon
2. Ferlucon
3. Ferronicum
4. Gluco-Ferrum
5. Iromin (Gador)
6. Irox
7. Nionate

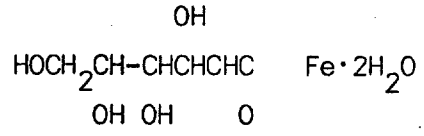
D. Chemical Abstracts Services Unique Registry Number

000 299 296

II. Empirical Formula

$C_{12}H_{22}FeO_{14} \cdot 2H_2O$

III. Structural Formula



IV. Molecular Weight

482.18

V. Specifications

Food Chemicals Codex	
Assay	Not less than 95.0% of $C_{12}H_{22}FeO_{14}$ calculated on dried basis
Loss on drying	Between 6.5 and 10%
Limits of impurities	
Arsenic (as As)	Not more than 3 ppm (0.0003%)
Chloride	Not more than 700 ppm (0.07%)
Ferric iron	Not more than 2%
Lead	Not more than 10 ppm (0.001%)
Mercury	Not more than 3 ppm (0.0003%)
Oxalic acid	Passes test
Reducing sugars	Passes test
Sulfate	Not more than 0.1%

VI. Description

A. General Characteristics

Yellowish gray or pale greenish yellow; slight odor of caramel or burnt sugar; acid to litmus.

B. Physical Properties

Occurs as a powder or granules; soluble in water, practically insoluble in alcohol.

C. Stability

The solid gluconate should be stored in tight containers; aqueous solutions are stabilized by addition of glucose.

VII. Analytical Methods (Food Chemicals Codex)

For assay, dissolve about 1.5 grams, accurately weighed, in a mixture of 75 ml of water and 15 ml of diluted sulfuric acid T.S. in a 300-ml Erlenmeyer flask, and add 250 mg of zinc dust. Close the flask with a stopper containing a Bunsen valve, allow to stand at room temperature for 20 minutes, then filter through a Gooch crucible containing an asbestos mat coated with a thin layer of zinc dust, and wash the crucible and contents with 10 ml of diluted sulfuric acid T.S., followed by 10 ml of water. Add orthophenanthroline T.S., and titrate the filtrate in the suction flask immediately with 0.1 N ceric sulfate. Perform a blank determination and make any necessary correction. Each ml of 0.1 N ceric sulfate is equivalent to 44.62 mg of $C_{12}H_{22}FeO_{14}$.

To identify:

A. To 5 ml of a warm 1 in 10 solution of the sample, add 0.65 ml of glacial acetic acid and 1 ml of freshly distilled phenylhydrazine, and heat the mixture on a steam bath for 30 minutes. Cool, and scratch the inner surface of the container with a glass stirring rod. Crystals of gluconic acid phenylhydrazide form.

B. A 1 in 20 solution gives positive tests for Ferrous salts.

VIII. Occurrence

Ferrous gluconate is suitably prepared for medical use by flavoring with about 20% syrup of orange with 0.3% citric acid added.

IRON LACTATE

Chemical Information

I. Nomenclature

A. Common Names

1. Iron Lactate
2. Ferrous Lactate

B. Chemical Name

Ferrous Lactate

C. Trade Names

None

D. Chemical Abstracts Services Unique Registry Number

005 905 522

II. Empirical Formula

$Fe(C_3H_5O_3)_2$

III. Structural Formula

Not applicable

IV. Molecular Weight

233.99

V. Specifications

None (C.P. as Food supplement)

VI. Description

A. General Characteristics

The trihydrate, $Fe(C_3H_5O_3)_2 \cdot 3H_2O$ is a greenish-white powder or crystalline mass with a slight characteristic odor and a sweet, ferruginous taste.

B. Physical Properties

Soluble in water and freely soluble in alkali citrates forming a green solution; almost insoluble in alcohol; exposure to air darkens the lactate and renders it less soluble.

C. Stability

Should be kept tightly closed, away from light.

VII. Analytical Methods (Food Chemicals Codex)

General qualitative tests for ferrous salts and lactate may be used.

VIII. Occurrence

None

Chemical Information

I. Nomenclature

A. Common Names

1. Iron Oxide
2. Ferrous Oxide
3. Ferric Oxide
   Ferric Sesquioxide
   Jewelers Rouge
   Hematite
   Magnetite

B. Chemical Names

1. Ferrous Oxide
2. Ferric Oxide

C. Trade Names

Siderac (ferrous-ferric oxide)

D. Chemical Abstracts Services Unique Registry Number

001 345 251 (Ferrous)
001 309 372 (Ferric)

II. Empirical Formula

FeO (ferrous)
Fe_2O_3 (ferric)
Fe_3O_4 (magnetite)

III. Structural Formula

Not applicable

IV. Molecular Weight

FeO - 71.85
Fe_2O_3 - 159.70

V. Specifications

None (C.P. as food supplement)

VI. Description

A. General Characteristics

Ferrous oxide is a jet-black powder; readily oxidizes in air; strong base, readily absorbs CO_2.

Ferric oxide occurs in three polymorphic forms, designated alpha, delta and gamma. The color and appearance depend upon the size and shape of the particles and amount of combined H_2O; typically red or black.

B. Physical Properties

Ferrous oxide melts at 1420°, is insoluble in water and alkalies, readily soluble in acids.

Ferric oxide decomposes at 1560°, is insoluble in water, soluble in H_2O. An 'active' magnetic form of ferric oxide was manufactured in Germany in the 1920's under the trade name SIDERAC.

C. Stability

Ferrous oxide should be stored in tightly closed containers.

VII. Analytical Methods

General qualitative tests for Fe may be used.

VIII. Occurrence

Ferric oxide occurs in nature as mineral hematite and magnetite (Fe_3O_4).

IRON PHOSPHATE

Chemical Information

I. Nomenclature

A. Common Names

1. As minerals: beraunite, cacoxenite, dufrenite, koninckite, phosphosiderite, strengite
2. Iron Phosphate
3. Ferric Phosphate
4. Ferric Orthophosphate

B. Chemical Name

Ferric Phosphate

C. Trade Names

None

D. Chemical Abstracts Services Unique Registry Number

010 045 871

II. Empirical Formula

$FePO_4 \cdot xH_2O$

III. Structural Formula

Not applicable

IV. Molecular Weight

150.82 (anhydride)

V. Specifications

Food Chemicals Codex	
Assay	Not less than 26.0% and not more than 30.0% of Fe
Loss on ignition	Not more than 32.5%
Limits on impurities	
Arsenic (as As)	Not more than 3 ppm (0.0003%)
Fluoride	Not more than 50 ppm (0.005%)
Lead	Not more than 10 ppm (0.001%)
Mercury	Not more than 3 ppm (0.0003%)

VI. Description

A. General Characteristics

Ferric phosphate is an odorless, yellowish-white to buff powder. The dihydrate, $FePO_4 \cdot 2H_2O$ is white, grayish-white, or light pink orthorhombic or monoclinic crystals or amorphous powder.

B. Physical Properties

Both forms are insoluble in water, but soluble in mineral acids. The dihydrate loses water above 140^{o}.

C. Stability

Store in tightly closed containers.

VII. Analytical Methods (Food Chemicals Codex)

Dissolve 1 gram in 5 ml of dilute hydrochloric acid (1 in 2), and add an excess of sodium hydroxide T.S. A reddish brown precipitate forms. Boil the mixture, filter to remove the iron, and strongly acidify a portion of the filtrate with hydrochloric acid. Cool, mix with an equal volume of magnesia mixture T.S., and treat with a slight excess of ammonia T.S. An abundant white precipitate forms. This precipitate, after being washed, turns greenish yellow when treated with a few drops of silver nitrate T.S.

VIII. Occurrence

Ferric phosphate may be manufactured by the reaction of ferrous sulfate, sulfuric acid and sodium chlorate. The ferric sulfate formed is treated with sodium phosphate dibasic and the resulting ferric phosphate is filtered, washed thoroughly with water, and dried.

IRON PYROPHOSPHATE

Chemical Information

I. Nomenclature

A. Common Names

1. Iron Pyrophosphate
2. Ferric Pyrophosphate

B. Chemical Name

Ferric Pyrophosphate

C. Trade Names

None

D. Chemical Abstracts Services Unique Registry Number

010 058 443
001 332 963 (soluble)

II. Empirical Formula

$Fe_4(P_2O_7)_3 \cdot xH_2O$

III. Structural Formula

Not applicable

IV. Molecular Weight

745.22 (anhydrous)

V. Specifications

Food Chemicals Codex	
Assay	Not less than 24.0% and not more than 26.0% of Fe
Loss on ignition	Not more than 20%
Limits of impurities	
Arsenic (as As)	Not more than 3 ppm (0.0003%)
Lead	Not more than 10 ppm (0.001%)
Mercury	Not more than 3 ppm (0.0003%)

VI. Description

A. General Characteristics

The common form of ferric pyrophosphate is the nonahydrate $Fe(P_2O_7)_3 \cdot 9H_2O$. It is a tan or yellowish-white, odorless powder.

B. Physical Properties

Insoluble in water, but soluble in mineral acids.

C. Stability

Should be stored in well closed containers.

VII. Analytical Methods (Food Chemicals Codex)

Dissolve 500 mg in 5 ml of dilute hydrochloric acid (1 in 2) and add an excess of sodium hydroxide T.S. A reddish brown precipitate forms. Allow the solution to stand for several minutes, and then filter discarding the first few ml. To 5 ml of the clear filtrate add 1 drop of bromophenol blue T.S., and titrate with 1 N hydrochloric acid to a green color. Add 10 ml of a 1 in 8 solution of zinc sulfate, and readjust the pH to 3.8 (green color). A white precipitate forms (distinction from orthophosphates).

VIII. Occurrence

None

IRON, REDUCED

Chemical Information

I. Nomenclature

A. Common Name

Reduced Iron

B. Chemical Name

Iron, Reduced

C. Trade Names

None

D. Chemical Abstracts Services Unique Registry Number

MX8 011 798

II. Empirical Formula

Fe

III. Structural Formula

Not applicable

IV. Atomic Weight

55.85

V. Specifications

Food Chemicals Codex	
Assay	Not less than 26.0% of Fe
Limits of impurities	
Acid-insoluble substances	Not more than 1.25%
Arsenic (as As)	Not more than 8 ppm (0.0008%)
Lead	Not more than 25 ppm (0.0025%)
Mercury	Not more than 5 ppm (0.0005%)

VI. Description

A. General Characteristics

Reduced iron is grayish-black, lusterless or only slightly so; amorphous powder, free of crystalline particles and fine enough to pass through a 100-mesh sieve.

B. Physical Properties

Reduced iron is soluble in dilute mineral acids, producing hydrogen and forming ferrous salts.

C. Stability

The iron is stable in dry air, but should be kept in well closed containers.

VII. Analytical Methods

Qualitative reactions producing hydrogen and ferrous salts which can be determined may be used.

VIII. Occurrence

Iron, reduced is manufactured by heating ferric oxide to a dull redness in a stream of dry hydrogen; or by decomposition of iron pentacarbonyl. (A purer form of elemental iron is produced electrolytically (electrodeposition); both forms are used as food additives.)

IRON SODIUM PYROPHOSPHATE

Chemical Information

I. Nomenclature

A. Common Names

1. Sodium Iron Pyrophosphate
2. Iron Sodium Pyrophosphate
3. Ferric Sodium Pyrophosphate
4. Sodium Ferric Pyrophosphate

B. Chemical Name

1. Ferric Sodium Pyrophosphate
2. Sodium Ferric Pyrophosphate

C. Trade Names

None

D. Chemical Abstracts Services Unique Registry Number

010 045 871

II. Empirical Formula

$Na_8Fe_4(P_2O_7)_5 \cdot xH_2O$

III. Structural Formula

Not applicable

IV. Molecular Weight

1277.00 (anhydrous)

V. Specifications

Food Chemicals Codex	
Assay	Not less than 14.5% and not more than 16.0% of Fe
Loss of ignition	Not more than 8%
Limits of impurities	
Fluoride	Not more than 50 ppm (0.005%)
Lead	Not more than 10 ppm (0.001%)
Mercury	Not more than 3 ppm (0.0003%)

Additional information from the NAS/NRC questionnaire indicates that a commercial product meets the above specifications plus:

Assay	Phosphorous pentoxide 49.3% maximum
Loss on drying	0.5% maximum
pH (5% suspension)	7.2-7.7

Must pass 99.0% minimum through screen, 325 mesh, USS.

VI. Description

A. General Characteristics

Sodium ferric pyrophosphate is a white to tan, odorless powder.

B. Physical Properties

Insoluble in water, soluble in HCl.

C. Stability

Should be stored in well-closed containers.

VII. Analytical Methods (Food Chemicals Codex)

Dissolve 500 mg in 5 ml of dilute hydrochloric acid (1 in 2), and add an excess of sodium hydroxide T.S. A reddish brown precipitate forms. Age the solution for several minutes, and then filter, discarding the first few ml. To 5 ml of the clear filtrate add 1 drop of bromophenol blue T.S., and titrate with 1 N hydrochloric acid to a green color. Add 10 ml of a 1 in 8 solution of zinc sulfate, and readjust the pH to 3.8 (green color). A white precipitate forms (distinction from orthophosphates).

VIII. Occurrence

The commercial pyrophosphate is manufactured from ferrous sulfate; phosphoric acid from elemental phosphorous; sodium carbonate or hydroxide, sulfuric acid and sodium hypochlorite.

IRON SULFATE
(Ferric)

Chemical Information

I. Nomenclature

A. Common Names

1. As Mineral, Coquimbite
2. Ferric Persulfate
3. Ferric Sesquisulfate
4. Ferric Tersulfate
5. Iron Sulfate

B. Chemical Name

Ferric Sulfate

C. Trade Names

None

D. Chemical Abstracts Services Unique Registry Number

010 028 225

II. Empirical Formula

$Fe_2(SO_4)_3$

III. Structural Formula

Not applicable

IV. Molecular Weight

399.88

V. Specifications

Commercial product usually contains about 20% H_2O and is yellowish in color. It is of little interest as a food additive.

VI. Description

A. General Characteristics

Grayish-white to brownish-yellow in color; crystalline or powder.

B. Physical Properties

Hydrates are very hygroscopic; soluble in cold water but decompose in hot water; slightly soluble in alcohol, insoluble in acetone and ethyl acetate.

C. Stability

Tight containers are required for storage and should be protected from light.

VII. Analytical Methods (Food Chemicals Codex)

General tests for ferric salts and for sulfates may be used.

VIII. Occurrence

Occurs in nature as the mineral coquimbite.

IRON SULFATE
(Ferrous)

Chemical Information

I. Nomenclature

A. Common Names

1. As anhydride minerals:

melanterite
siderotil
szomolnikite
tauriscite

2. As hydrate:

dried ferrous sulfate
exsiccated ferrous sulfate

3. As heptahydrate:

copperas
green vitriol
iron vitriol

B. Chemical Name

1. Ferrous Sulfate

C. Trade Names

1. As hydrate:

Feromax
Ferro-Gradumet

2. As heptahydrate

Feosol
Fesofor
Haemofort
Ironate
Irosul
Presfersul
Sulferrous

D. Chemical Abstracts Services Unique Registry Number

007 782 630
010 028 214 (Dihydrate)
007 782 630 (Heptahydrate)
007 720 787 (Anhydrous)
977 001 447 (Dried)

II. Empirical Formula

$FeSO_4$

III. Structural Formula

Not applicable

IV. Molecular Weight

151.91

V. Specifications ($FeSO_4 \cdot 7H_2O$)

Food Chemicals Codex	
Assay	Not less than 99.5% and not more than the equivalent of 104.5% of $FeSO_4 \cdot 7H_2O$
Limits of impurities	
Arsenic (as As)	Not more than 3 ppm (0.0003%)
Lead	Not more than 10 ppm (0.001%)
Mercury	Not more than 3 ppm (0.0003%)

VI. Description

A. General Characteristics

The hydrate is a white to yellow crystalline powder; the heptahydrate is blue-green monoclinic crystals or granules. Both are odorless.

B. Physical Properties

The hydrate loses the H_2O at about 300°, decomposes at higher temperatures. It is soluble in water.

The heptahydrate is efflorescent in dry air, oxidizes in moist air to form brown coating of basic ferric sulfate. It is soluble in water, insoluble in alcohol.

C. Stability

Ferrous sulfate should be kept in tightly closed containers, away from light.

VII. Analytical Methods (Food Chemicals Codex)

A test for ferrous sulfate consists of dissolving about a gram (accurately weighed) of the sample in 25 ml of diluted sulfuric acid test solution and 25 ml of recently boiled and cooled water. This is titrated with 0.1 N potassium permanganate until a permanent pink color results. Each ml of 0.1 N potassium permanganate is equivalent to 27.80 mg of $FeSO_4 \cdot 7H_2O$.

VIII. Occurrence

The anhydride occurs in nature as minerals (see Common Names).

Biological Data

I. Acute toxicity

Substance	Animal	No.	Route	Dosage (mg/kg Body Wt.) Fe	Measurement	Ref.
Iron Ammonium	Mouse		i.v.	16.5	LD_{50}	146
Citrate	Mouse		oral	1000.0	LD_{50}	146
	Guinea pig		oral	350.0	LD_{50}	146
	Rabbit		oral	560.0	LD_{50}	146
Iron Carbonate	Mouse		oral	3800.0	LD_{50}	146
	Guinea pig		oral	2000.0	LD_{50}	146
	Rabbit		oral	2220.0	LD_{50}	146
Iron Chloride	Mouse		i.v.	18.5	LD_{50}	146
(ferric)	Mouse		oral	500.0	LD_{50}	146
	Mouse		oral	840.0	LD_{50}	146
	Mouse		i.v. slowly	.049 (mg Fe/g)	LD_{50}	149
	Mouse		oral	.44 (mg Fe/g)	LD_{50}	149
	Guinea pig		oral	200.0	LD_{50}	146
	Rabbit		oral	400.0	LD_{50}	146
	Dog		oral	3.75-5 gm*	Fatal, 27-30 hrs	146
	Dog		oral	2.5 gm*	Severe illness	146
Iron Chloride	Rat		oral	14.0	Somewhat ill	146
(ferrous)	Rat		oral	18.0	Somewhat ill	146
	Rat		oral	28.0	LD_{50}, 24 hrs	146
	Rat		oral	56.0	LD_{100}, 1/2-30 hr	146
	Rat		rectal	28.0	LD_{100}, 48 hrs	146
	Rat		rectal	56.0	LD_{100}, 5 min	146
	Rabbit		oral	168.0	No effect	146
	Rabbit		oral	224.0	No effect	146
	Rabbit		oral	252.0	Fatal, 24 hrs	146
	Rabbit		oral	280.0	Fatal, 24-48 hr	146
	Rabbit		rectal	280.0	Fatal, 1/2-5 hr	146
Iron Gluconate	Mouse		oral	101 ± 52	LD_{50}	119
	Mouse		oral	1100.0	LD_{50}	146
	Mouse	55	i.v.	23.	LD_{50}	357

Substance	Animal	No.	Route	Dosage (mg/kg Body Wt.) Fe	Measurement	Ref.
	Mouse	100	oral	457.4	LD_{50}	357
	Rat	24	oral	865.0	LD_{50}	357
	Guinea pig		oral	350.0	LD_{50}	146
	Rabbit		oral	580.0	LD_{50}	146
	Dog	9	oral	46.4	LD_{50}	357
Iron Sulfate	Mouse		oral	900.0	LD_{50}	146
(ferrous)	Mouse		oral	1000.0	LD_{50}	146
	Mouse		oral	710.0	LD_{50}	146
	Mouse		i.v. rapidly	.013 (mg Fe/g)	LD_{50}	149
	Mouse		i.v. slowly	.028 (mg Fe/g)	LD_{50}	149
	Mouse		i.p.	.047 (mg Fe/g)	LD_{50}	149
	Mouse		oral	.15 (mg Fe/g)	LD_{50}	149
	Mouse	55	i.v.	33.0	LD_{50}	357
	Mouse	40	oral	305.0	LD_{50}	357
	Rat	24	oral	780.0	LD_{50}	357
	Guinea pig		oral	300.0	LD_{50}	146
	Guinea pig		oral	300.0	LD_{50}	146
	Guinea pig		oral	400.0*	Fatal, 18 1/2 hr	146
	Guinea pig		oral	200.0*	Fatal, 1/2 hr	146
	Guinea pig		oral	400.0*	Survived	146
	Guinea pig		oral	600.0*	Fatal	146
	Guinea pig		oral	800.0*	Fatal	146
	Rabbit		oral	600.0	LD_{50}	146
	Rabbit		oral	720.0	LD_{50}	146
	Rabbit		oral	368.0	ill, survived	146
	Rabbit		oral	736.0	Fatal	146
	Rabbit		oral	540.0	ill, survived	146
	Rabbit		oral	769.0	Fatal, 1 1/2 hr	146
	Rabbit		oral	1000.0*	No ill effects	146
	Rabbit		oral	1869.0	Fatal 1 hr	146
	Rabbit		oral	3000.0	Survived, plus $NaHCO_3$, 3 gm	146

Substance	Animal	No.	Route	Dosage (mg/kg Body Wt.) Fe	Measurement	Ref.
	Rabbit		oral	4327.0	Fatal, 3-4 hr	146
	Rabbit		oral	3000.0*	Fatal	146
	Cat		oral	240.0	Survived	146
	Dog		oral	930.0	ill, survived	146
	Dog		oral	2000.0*	ill, survived	146
	Dog		oral	8000.0*	Fatal, 26 hr	146
	Dog	16	oral	23.5	LD_{50}	357

*Total dose as salt

A table of acute iron toxicity in man is shown on page 38.

A. Iron ammonium citrate

Ferric ammonium citrate has become a common form of iron therapy, with only a few reported cases of acute toxicity. A young, pregnant woman, hoping to induce an abortion, was reported in the British Medical Journal in 1950 as having consumed some 15 gm of iron ammonium citrate in whiskey; she died of toxic hepatitis three days later. An older woman, received 10 gm of iron salt per day for 23 days in treatment for anemia, the dose was increased to 12.5 gm on the 24th day, whereupon the next morning she suffered severe vomiting and loss of consciousness, plus additional toxic symptoms. The therapy was stopped, and the woman gradually recovered (146).

B. Iron carbonate

None

C. Iron chlorides

Ferric chloride is of particular interest as the agent reported used in 4 homicides. As little as 6 gm of the salt proved fatal when taken orally by an adult male. As far back in time as 1884, a case of attempted murder with ferric chloride was recorded (146).

The chloride is listed in the Merck Index as an irritant, astringent and rarely used internally.

Ferrous chloride has been responsible in Sweden for at least 3 reported cases of toxicity, all very young children. A 2 1/2-year-old girl ingested about 20 tablets for a total of more than 5 gms of ferrous chloride, survived - but was in generally poor condition for some time; a similar case involved taking twice as much chloride with subsequent extensive necrosis of the stomach wall. A third case, a 17-month-old boy who swallowed an unknown number of his mother's anti-anemia iron

tablets, died in little more than a day later despite hospitalization and therapy (146).

D. Iron gluconate

Weaver et al. studied comparative toxicologies of various iron compounds including ferrous gluconate. The LD^{50} dose, orally for mice (100) was 3950 mg/kg as the salt, or 457.4 mg/kg of Fe; the LD^{50} for rats (24) orally was 7460 mg/kg as salt, 865 mg/kg as Fe; and the LD^{50} for dogs (9) was 400 mg/kg as salt, and is greater than 46.4 as Fe by intravenous injection. The gluconate administered orally by capsule with a dose of 400 mg/kg of the salt in five dogs produced emesis in two; a dose of 800 mg/kg caused emesis in all of five dogs (357).

Ferrous gluconate, shown to be the most readily absorbed form of ferrous iron salts, produces less gastric disturbance and is widely used in anemia therapy. Only one report of acute toxicity is found, even though treatments involving as much as a gram per day over a period of several months are reported.

Henderson et al. reported a case of a 14 1/2-month-old girl who ingested about thirty-two ferrous gluconate tablets (0.3 gm), becoming critically ill. A new specific iron chelating agent, desferrioxamine was used in therapy. The child recovered virtually completely within a month after discharge from the hospital (134).

E. Iron lactate

None

F. Iron oxides

None

G. Iron phosphate

None

H. Iron pyrophosphate

None

I. Iron reduced

None

J. Iron sodium pyrophosphate

None

K. Iron sulfate, ferric

None

L. Iron sulfate, ferrous

At present, the use of ferrous iron is widespread, with millions of iron pills dispensed annually. The tablets (usually ferrous sulfate) are typically brightly colored and sugar- or chocolate-coated, so that one is not surprised to learn that of 63 cases of reported poisonings, accidental and homicidal, of orally ingested ferrous sulfate, 21 children and two adults died (145). A typical case is described by Curtiss (64). Many of the nonfatal poisonings were of very young children, who were saved by prompt gastric lavage and supportive therapy. As few as a dozen or so of ferrous sulfate tablets proved fatal to a 19-month-old child and as few as eight produced severe reactions in a 2-year-old. Each tablet of one popular brand contains 200 mg of $FeSO_4$, with additional 2.6 mg of both copper sulfate and manganese sulfate. Neither the copper nor the manganese sulfates have been shown to contribute materially to the toxic action of the tablets (95). In both animals and humans, fatally poisoned by overdosage of iron, postmortem examinations show hemorrhagic gastritis with edema, leading apparently to fibrous contracture of the pyloric antrum and subsequent stenosis or blockage of the pylorus (293, 310). James O. Hoppe et al. who have reviewed the history of iron therapy (145) suggest that "lack of appreciation of the reality of ferrous sulfate poisoning by doctors and hospitals makes it necessary to emphasize that ferrous sulfate intoxication may be serious, and that immediate treatment is essential".

Estimates of the human lethal dose of iron preparations are, of course, difficult if not impossible. In a few instances, accurate information has been available regarding amounts of ferrous sulfate ingested, but in general only approximations are possible. Fatal doses of ferrous sulfate range from 40-1600 mg/kg, with average value of about 900 mg/kg (146). When this figure is compared with fatal doses in animal studies, it appears much smaller than those for oral toxicity values for mice (4500 mg/kg), guinea pig (1500 mg/kg) and rabbit (3000 mg/kg); figures found for cat (greater than 500 mg/kg) and dog (800 mg/kg) are quite similar. Special note must be taken of the fact that the value of the human fatal dose of about 900 mg/kg is largely based on cases of poisoning of children 2 years-old or younger.

Sommers in a report of the relative oral toxicity of some therapeutic iron preparations states; "It is reasonable, and probably a wise precaution, by extrapolation to accept as proved that all similar iron compounds can, in excessively large doses, kill mammals of any species, including man. It must, however, be emphasized that toxic doses really are excessive. The amount of ferrous sulfate necessary to kill on the average one out of two 10-stone (63.5 kg) men, if man's susceptibility on a body-weight basis is assumed to be the same as that of the rabbit, would represent at least several hundred tablets of 3 gr (0.2 g) of exsiccated ferrous sulfate, each containing 1 gr (65 mg) of iron. Obviously the number may be considerably smaller for infants and young children, being reckoned in tens rather than hundreds." (321).

Gimlett reports 3 cases of ferrous sulfate poisoning in children, one of which was fatal: a 3-year-old boy ingested approximately 80

tablets containing 24 gm ferrous sulfate, recovered completely in 48 hours following intensive hospital therapy; a 17-month-old girl ingested an unknown number of 300 mg tablets and recovered also after 48 hours of therapy; a 2-year-old Indian boy ingested an estimated 15 tablets, 300 mg each of ferrous sulfate, therapy was not effective and the boy died. Nine other similar cases were mentioned, one a fatality (111).

Clinical observations are reported of a 2-year-old boy who swallowed 40 ferrous sulfate tablets; hospitalized half an hour later and given intensive therapy, he slowly recovered. Pyloric stenosis a month later necessitated a gastroenterostomy (42). A similar case, involving a girl of 21 months who swallowed an unknown number of tablets, is reported by Wilmers and Herob (365). After ten days of hospitalization with typical symptoms, a posterior gastrojejunostomy was performed to relieve pyloric stenosis. The child recovered and later at the age of nearly 11 years was found to have developed normally. A 2-year-old boy suffered a similar experience, having ingested 40 tablets. A gastroenterostomy was performed, with recovery again.

A fatal case of poisoning with fewer than 20, 0.3 g ferrous sulfate tablets, in a 17-month-old girl is reported by Smith (319). The symptoms were vomiting, tarry stools, cyanosis and difficulty in breathing. The autopsy showed necrosis of the pharyngeal mucosa; bronchopneumonia; necrosis of esophagus, stomach and small intestine; and swelling of the brain.

Eight cases of ferrous sulfate poisoning are reported by Spencer (323). He describes the most readily available iron tablets as "green sugar-coated pills", each containing ferrous sulfate (3 gr), copper sulfate (1/25 gr) and manganese sulfate (1/25 gr), with the ferrous sulfate having the irritating and lethal effects. Four of the children poisoned recovered; four died. Each case is detailed and differs from others reported primarily in that the dose of iron was fairly well established.

Thomson (342) reports six cases of children having swallowed iron tablets like those described by Spencer. Two of the children died. The author suggests treatment in iron poisoning cases by gastric lavage with aqueous $NaHCO_3$ solution. He further points out, as have others, that ferrous sulfate is one of the most dangerous drugs commonly used and must be hidden securely from children. Thomson had earlier (341) reported two cases, one of whom died. The fatality resulted from ingesting about 26 tablets; the survivor took only eight.

Aldrich (6) describes the clinical features of acute iron toxicity in children and summarizes 42 case reports from the medical literature in the following table:

Acute Iron Toxicity

Age in months	Iron	Dosage Taken	Treatment	Duration of illness	Outcome
30	$FeSO_4$ tabs.	10 gm.	none	53 hours	fatal
12	$FeSO_4$ tabs.	6 gm.	supportive	30 hours	fatal
13	$FeSO_4$ tabs.	6 gm.	oxygen, lavage, transfusion	dismissed from hospital 8 days	recovered
16	$FeSO_4$ tabs.	5 gm.	lavage $NaHCO_3$	21 hours	fatal
21	$FeSO_4$ tabs.	1.6 gm.	supportive	dismissed from hospital 11 days	recovered
15	$FeSO_4$ tabs.	2.6 gm.	supportive	dismissed 4 days	recovered
20	$FeSO_4$ tabs.	16 gm.	O_2, transfuse	21 hours	fatal
21	$FeSO_4$ caps.	"large number"	transfuse	48 hours	fatal
18	$FeSO_4$ tabs.	"unknown"	transfuse	dismissed 11 days	recovered
19	$FeSO_4$ tabs.	2.0 gm.	lavage and supportive	36 hours	recovered
26	$FeSO_4$ caps.	13 gm.	lavage, Spencer formula, BAL, transfuse	dismissed 6 days	recovered
15	$FeSO_4$ tabs.	8.6 gm.	O_2 lavage	1 hr. 15 min.	fatal
21	$FeSO_4$ tabs.	"unknown"	lavage	surgery in 5 weeks	recovered after surgical repair of scar
21	$FeSO_4$ tabs.	8.0 gm.	lavage $NaHCO_3$	surgery in 5½ weeks	
54	$FeSO_4$ tabs.	7.2 gm.	vomiting, bismuth subcarb.	"short"	recovered
19	$FeSO_4$ tabs.	3.0 gm.	lavage with $NaHCO_3$, BAL	18 hours	recovered
30	$FeSO_4$ tabs.	1.5 gm.	lavage $MgSO_4$	dismissed 3 days	recovered
20	$FeSO_4$ tabs.	"unknown" (?) 18 gm.	transfusion, supportive	4½ hours	fatal
15	$FeSO_4$ tabs.	6.0 gm.	supportive	18 hours	recovered
18	$FeSO_4$ tabs.	1.5 gm.	plasma, lav.	dismissed 5 days	recovered
26	$FeSO_4$ tabs.	"unknown"	O_2 lavage	4½ hours	fatal
19	$FeSO_4$ tabs.	"unknown"	transfuse BAL, support.	30 hours	fatal
17	$FeSO_4$ tabs.	(?) 3 gm.	transfuse	acute illness for 13 days, surgery on 58th day	pyloric stenosis recovered after surgery
14	$FeSO_4$ tabs.	"unknown"	plasma, lavage $NaHCO_3$	acute 19 days, surgery 15th day.	recovery, postop.
30	$FeSO_4$ tabs.	15.0 gm.	saline lavage	dismissed 11 days	recovered
17	$FeSO_4$ tabs.	6.0 gm.	plasma, methylene blue, supportive	11 hours	fatal
16	$FeSO_4$ tabs.	"unknown"	transfusion	12 days acute illness, 60 days until surgery	recovered following partial gastrectomy for pyloric stenosis
16	$FeSO_4$ tab.	"unknown"	supportive	31 hours	fatal
18	$FeSO_4$ tabs.	"unknown"	lavage, supportive	20 hours	fatal
12	$FeSO_4$ tabs.	"unknown"	supportive	1 hour	fatal
11	$FeSO_4$ tabs.	15-20 gm.	BAL, lavage, fluid	dismissed 9 days	recovered
21	$FeSO_4$ tabs.	15.0 gm.	lavage $NaHCO_3$	dismissed 11 days	recovered, mental signs only while sick
23	$FeSO_4$ tabs.	6.5 gm.	supportive	dismissed 6 days	recovered
11	$FeSO_4$ tabs.	3.9 gm.	lavage	dismissed 3 days	recovered
20	$FeSO_4$ tabs.	4.5 gm.	none	dismissed 24 hours	recovered
12	$FeSO_4$ tabs.	"unknown"	castor oil	5 hours	fatal
13	$FeSO_4$ tabs.	4.8 gm.	none	4 hours	fatal
18	$FeSO_4$ tabs.	13.2 gm.	lavage	5½ hours	fatal
11	$FeSO_4$ tabs.	13.2 gm.	none	20 hours	fatal
24	FeCL	5.4 gm.	transfusion	dismissed 2½ months	recovered, slight gastric changes
20	$FeSO_4$ tabs.	10 gm.	transfuse, lavage	16 weeks	fatal, stricture gastric
21	$FeSO_4$ tabs.	8.2 gm.	lavage $NaHCO_3$	4 hours	fatal

A case report by Smith (317) involving a male child age 21 months who presumably swallowed about 40 ferrous sulfate tablets and despite treatment for profound shock expired 4 hours after ingesting the tablets. Autopsy findings are described and histological appearances detailed. The role of ferritin in iron absorption and production of the shock syndrome is discussed.

A fatal case of ferrous sulfate poisoning is reported by Branch (39), involving a 29-month-old boy who swallowed more than 60 and possibly up to 75, 300 mg tablets (a dose of about 1.87 gm/kg body weight). Following gastric lavage he appeared recovered; an hour later he was readmitted to the hospital, violently ill, and died within one hour, despite emergency treatment. The characteristics of vomiting, hematemesis, tarry stools, vasomotor collapse and cyanosis found in other such cases were present. Postmortem observations included hemorrhagic gastroenteritis with mucosal slough and submucosal venous thromboses.

Five cases of ferrous sulfate poisoning in children ranging in age from 18 months to 3 years are described by Emmanouilides (86). One patient died, one severely affected and three mildly affected. In each case, the number of iron pills ingested was not determined. The clinical picture, pathological findings, pathogenesis, treatment and prevention of iron poisoning are discussed.

Burrows (49) reports a severe ferrous sulfate poisoning case of a child 14 months old having swallowed about 20 tablets. After nine hours of hospital therapy the boy recovered.

Two cases of pyloric obstruction caused by ferrous sulfate poisoning in infants are reported by Forshall and Rickham (96). Both infants recovered following a posterior no-loop gastrojejunostomy in the one case, and a pyloroplasty in the other. A review of similar cases of pyloric obstruction concludes the report.

Hoyt (152) reports a case, non-fatal, of a child of 19 months swallowing about 10 ferrous sulfate pills. She recovered after hospitalization for 36 hours. The author suggests that the possibility of poisoning by this widely used drug should be more generally known to physicians and that physicians and druggists alike, should take steps to prevent its occurrence.

A brief review of reported ferrous sulfate poisonings is followed by a typical case history and a discussion of various treatments by Murphy et al. (238). Again, the dangers of ferrous sulfate are emphasized.

Jaco and Pugh (156) describe a case of a 15-month-old child, fatally poisoned by about 43 'Fersolate' (ferrous sulfate) tablets. A very detailed postmortem autopsy is included in the report.

A report by Covey (61) is prefaced by a brief review of the history of ferrous sulfate poisoning, followed by four case histories of children, one fatal poisoning and three severe cases. The value of Edathamil calcium disodium EDTA in therapy of iron poisoning is discussed and compared with various other forms of treatment, e.g. exchange

transfusion; combined chelation; hemodialysis and alkalinization; intravenous calcium disodium EDTA and hemodialysis; and peritoneal dialysis. Four phases of reaction to severe acute ferrous sulfate poisoning are noted: hemorrhagic gastroenteritis; delayed profound shock; liver injury, and gastric obstruction. A fifth possible phase, cirrhosis due to subfatal liver damage, has not as yet (1964) been reported.

Kaplan and Schliefer (166) describe a typical case of ferrous sulfate poisoning in an infant of 13 months and recommend adequate labeling of the drug as a potential poison.

Birk et al. (31) present a case report of acute ferrous sulfate poisoning in a 26-month-old female infant with complete recovery. A brief review of the literature covering the late 1940's and early 1950's is presented in the following table:

AUTHOR	CASE NO.	AGE (MO.)	SEX	DOSE (GM.)	TREATMENT	RESULT
Branch[31]	1	29	M	18.0-22.5	Lavage, O_2, heat, suction, IV fluid, blood	Died, 4½ hr.
Duffy and Diehl	2	15	F	4.9-6.4	IV saline	Recovered
	3	18	M	4.9	Lavage, IV fluid, plasma, blood, penicillin	Recovered
	4	26	F	9.75-13.0	Lavage, heat, O_2, IV fluid, caffeine	Died, 4½ hr.
Forbes[135]	5	39	M	10.0*	None	Died, 53 hr.
	6	12	M	6.0-7.0*	Heat, milk, Nepenthe, O_2, atropine, penicillin	Died, 30 hr.
Foucar[97]	7	26 yr.	M	113.5	Lavage, O_2, blood, artificial respiration	Died, 3 hr.
Lancet	8	16	F	8.0*	?	Died
Lindquist	9	24	F	5.34†	Blood, penicillin	Recovered
	10	7	?	10.68†	?	Died
Murphy[238]	11	30	F	15.0	Lavage, Na bicarbonate, AlOH gel, penicillin, milk	Recovered
Prain	12	11	F	?*	Lavage, Na bicarbonate, sulfa, bismuth carbonate	Died, 39 hr.
Roxburgh	13	16	M	6.0-9.75	Lavage, $MgSO_4$, IV fluid, penicillin, BAL	Recovered
Shoss[310]	14	14	F	16.3-24.4	Lavage, IV fluid, milk, O_2, penicillin, BAL	Recovered
Smith, J.[317]	15	21	M	8.2*	Lavage, Na bicarbonate	Died, 4 hr.
Smith, R.[316]	16	17	F	6.5?	Coramine, O_2, plasma, methylene blue, IV fluid	Died, 11 hr.
Spencer[342]	17	21	F	10.8*	Lavage, Na bicarbonate, bismuth carbonate (serum iron: 4 hr., 3.3 mg.; 3 days, 0.26 mg.)	Recovered
	18	23	M	5.8*	Saline, bismuth carbonate, vitamins,‡ IV fluid (serum iron: 4½ hr., 3.42 mg.; 6 days, 0.33 mg.)	Recovered
	19	11	M	1.4-1.8*	Lavage, bismuth carbonate, vitamins‡	Recovered
	20	20	M	0.6*	None	Recovered
	21	12	M	?*	Saline, castor oil	Died, 4 hr.
	22	19	F	3.0-3.2*	Saline	Died, 4 hr.
	23	18	M	8.8*	Lavage, stimulants	Died, 5½ hr.
	24	14	F	8.0*	Castor oil, kaolin	Died, 20 hr.
Swift	25	19	F	?	IV fluid, blood, BAL, Penicillin, streptomycin, O_2, vitamin K, Amphojel	Died, 40 hr.
Thomson[340]	26	16	F	5.2*	Lavage, Na bicarbonate, Nepenthe, bismuth carbonate	Died, 21 hr.
	27	24	M	1.6*	Magnesium hydroxide, bismuth mist., IV fluid, milk	Recovered
Thomson[342]	28	51	F	0.8*	Bismuth carbonate	Recovered
	29	19	M	2.0*	Syrup of figs, lavage, Na bicarbonate, BAL	Recovered
	30	30	M	2.0-4.0*	Syrup of figs, lavage, magnesium sulfate	Recovered

*Also 12.5 mg. copper sulfate and 12.5 mg. manganese sulfate per gram of ferrous sulfate.
†Ferric chloride.
‡See text.

An early (1948) case report of ferrous sulfate poisoning fatality by Foucar et al. (97) is rather unique in that it involved an adult male, age 26, who ingested accidentally one-quarter pound of the compound in aqueous suspension. Death occurred within three hours. Severe gastrointestinal irritation was observed, with death attributed to shock. There was no evidence, clinical, pathologic or toxicologic - of absorption of the ferrous sulfate.

Two cases with characteristic symptoms of iron poisoning are described by Clark et al. (57); one was fatal, the other child recovered. Where X-rays reveal iron tablets ingested and present as a group in the intestine, a laparotomy should be considered, after recovery from the initial shock, to remove the tablets and to resect necrotic portions of the bowel.

The use of British anti-lewisite (BAL) in therapy of a case of severe ferrous sulfate poisoning in a 14-month-old white female with a favorable outcome is described by Shoss (311). Further therapeutic trials of BAL in similar cases is suggested by the author.

II. Short Term Studies

A. Iron ammonium citrate

None

B. Iron carbonate

Dogs

Mongrel dogs of either sex, weighing 5-16 kg, were given commercial preparations of ferrous carbonate, sulfate and gluconate in a study by D'Arcy and Howard. The dogs were fed daily a regular diet of dog food, biscuits and tap water, supplemented by the iron salts, for a period of 14 consecutive days. The animals were sacrificed on the day following the last dose and sections of stomach and intestine were examined histologically. Eight of the dogs received doses of ferrous carbonate varying from 0.125 mg Fe/kg body weight to 1.0 gm/kg. None showed distress during the experimental treatment nor was there post-mortem evidence of gastrointestinal damage with the exception of a single animal slightly affected. Similar treatments with ferrous sulfate in six dogs resulted in several cases of vomiting and postmortem damage; the group of six dogs fed the gluconate showed some discomfort, one vomiting and one showing some gastrointestinal damage. With the single exception noted, doses of from 0.25 gm of Fe++/kg to 1.0 gm of Fe++/kg in the form of ferrous carbonate produced no symptoms of toxicity nor post-mortem evidence of gastrointestinal damage. The authors also studied the therapeutic value of the carbonate in treating anemic piglets and found it quite effective (70).

C. Iron chlorides

None

D. Iron gluconate

Mice

A study has been made of the relative gastrointestinal irritation produced by the oral ingestion of ferrous gluconate in syrup form and an iron-carbohydrate complex; the relative therapeutic efficacies were also observed. LD_{50} studies in mice were performed by fasting the mice (numbers and details not given) for about 20-24 hours, then orally intubating the two iron compounds with doses calculated on milligram/kilogram weight basis. The number of animals dead after 24 hours was observed. The LD_{50} of ferrous gluconate was 101 ± 52 mg/kg; the LD_{50} of the complex could not be determined as the doses proved to large for oral feeding (119).

The toxicity of ferrous gluconate relative to ferrous sulfate was studied by administering both salts intravenously or orally in mice (145). The results indicate a toxicity of ferrous gluconate about half that of the sulfate, on the basis of the salts; on the basis of Fe alone, there is little difference when administered intravenously. The following table summarizes the experiment on mice:

ACUTE TOXICITY OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) VERSUS FERROUS GLUCONATE ($Fe(C_6H_{11}O_7)_2 \cdot 2H_2O$) IN MICE

Compound	Route of Admin.	No. of Animals	LD_{50} ± s.e. mg./kg. As Salt 24 Hours	As Salt 7 Days	As Fe^{++} 24 Hours	As Fe^{++} 7 Days
Ferrous sulfate	I.V.	30	65 ± 4.8	51 ± 4.6	18 ± 1	10.2 ± 0.9
Ferrous gluconate	I.V.	40	114 ± 7.6	98 ± 6.8	12.5 ± 0.7	10.8 ± 0.7
Ferrous sulfate	Oral	30	1520 ± 130	1520 ± 130	306 ± 26	306 ± 26
Ferrous gluconate	Oral	60	3700 ± 145	3700 ± 145	429 ± 17	429 ± 17

Rats

Ferrous gluconate toxicity, related to that of ferrous sulfate, was determined by oral administration in rats, and the LD_{50} observed. A low toxicity characterizes the gluconate which ranks only a third as toxic as the sulfate, in salt form, or about half as toxic as Fe (146).

Thirty animals each were given the sulfate ($FeSO_4 \cdot 7H_2O$) or the gluconate $Fe(CuH_{11}O_4)_2 \cdot H_2O$ and observed at 24 hours and at 7 dats. As salt, the LD_{50} for the sulfate was 1480 mg/kg; as Fe, 518 mg/kg; for the 7-day LD_{50}, as salt, 4500 mg/kg; as Fe, 507 mg/kg.

Rabbits

Serum iron levels were estimated in 2 groups of rabbits, seven in each group, before and after administering the iron compounds. The gluconate and the carbohydrate complex were essentially equally effective in producing a rise in serum iron (119).

Twelve albino rabbits (1.2-1.5 kg) were made anemic by bleeding and divided into two groups after serum iron and hemoglobin were determined: the first group received the iron-carbohydrate complex in daily doses of 30 mg Fe/kg body weight for three weeks; the second group received an identical dose of gluconate syrup for the same period of time. Again, both forms of iron dosage showed nearly similar increases in serum iron levels and percent of hemoglobin (119).

Rabbits were used in a study of the local tissue toxicity of ferrous gluconate and sulfate estimated by the trypan blue irritation test. A mild irritation is indicated by a faint but discernible blue color at the site of the injection first of the ferrous salts into the abdominal skin of the animal, followed by intravenous injection of 10 mg/kg of trypan blue. For the sulfate a concentration in percent, as salt, of 0.25 showed mild irritation, 0.5 showed moderate irritation, and 1.0 to 2.0 was markedly irritating. For the gluconate, a concentration of 1.0 was mild, 2.0 was moderate and 4.0-8.0 was markedly irritating (146).

Cats

The relative toxicities in cats of ferrous gluconate and sulfate were studied in groups of four animals by oral administration of the two iron salts (145). The emetic effect of large doses in cats prevented determination of the LD_{50}, but the effects of repeated large doses were observed. Intense gastric irritation prevented estimates of acute oral toxicity because of prompt and copious vomiting, though about twice as much gluconate was tolerated over the sulfate before vomiting occurred. Neither mortality nor cumulative toxicity in cats resulted from daily oral doses, 5 days a week for 2 weeks, at excessive doses of 100-1600 mg/kg of the gluconate. Emesis and diarrhea were observed at all levels of dosage.

Dogs

Comparison of the relative toxicity of ferrous gluconate and ferrous sulfate in dogs was studied by single oral dosages of the sulfate ranging from 50-800 mg/kg of the salt and 100-3200 mg/kg of the gluconate as salt. Emesis was observed only at higher doses, but diarrhea was noted at all levels. The acute oral median lethal dose in dogs was estimated to exceed 800 mg/kg of ferrous sulfate, and more than 3200 mg/kg of the gluconate (146).

Man

The relative therapeutic efficacies of ferrous gluconate in syrup form, and an iron-carbohydrate complex, by oral ingestion in selected hospital patients was the subject for several experiments.

Eight normal healthy adults were given the carbohydrate complex containing 100 mg Fe orally; serum iron was estimated both in the fasting state and 3 hours after dosage. Fifteen days later an equivalent amount of the gluconate syrup was given, and serum iron determined. The percentage rise in the complex was 97.5%, the ferrous gluconate, 98.5%.

Forty male anemic patients were given orally the syrup and the complex in separate groups of 20 each in doses of 100 mg Fe daily,

weekly hemograms were carried out and the two groups compared for relative therapeutic value. After 4 weeks, the hemoglobin levels and packed cell volumes were essentially the same.

The authors conclude that inasmuch as the iron-carbohydrate complex is as biologically effective as the iron salt and far less apt to produce gastrointestinal upset, the complex is a more desirable form of iron in therapy (119).

E. Iron lactate

None

F. Iron oxides

None

G. Iron phosphate

None

H. Iron pyrophosphate

None

I. Iron reduced

Rats

Shanas and Boyd (308) have reviewed the centuries-old history of the medicinal use of powdered iron (iron reduced, in modern terminology), noting its fall from favor with the introduction in modern times of numerous iron-containing preparations. The absence of any reference in the literature to toxicity of reduced iron led to experiments using albino rats, normal and nonanemic, orally administered reduced iron powder in groups of 10-15 animals each, by means of an intragastric tube. The iron was given as a suspension in water, in volumes of 75 ml/kg body weight, in doses ranging from 0-200 gm/kg body weight. The LD_{50} was found to be 98.6 ± 26.7 gm/kg body weight. No animals died from doses less than 50 gm/kg. Comparison with other iron sources is shown in the following table:

The Median Lethal Dose of Iron Given Orally to Albino Rats as Various Salts

Preparation	LD_{50},[a] gm/kg
Iron carbohydrate complex	4
Ferrous sulfate	1
Ferrous chloride	1
Ferrous gluconate	1
Ferric chloride	0.4
Ferrous fumarate	0.3
Reduced iron	100

[a]The results are expressed as elemental iron and are reduced to one significant figure.

The doses of 100 gm of iron powder/kg or greater produced death chiefly by obstructing the bowel. Maximum LD_{50} was at 50 gm/kg in the rat, which would correspond to a child of 15 kg body weight ingesting 4000, 200 mg capsules of iron powder. Toxicity-wise, iron powder would appear the drug of choice in anemia therapy.

J. Iron sodium pyrophosphate

None

K. Iron sulfate, ferric

None

L. Iron sulfate, ferrous

Mice

Mature, female albino mice were given a wide variety of doses of ferrous sulfate in groups of 5 or 10 animals and the LD_{50} determined for various means of administration of the sulfate. The results are shown in the following table:

Results of the Investigation

Iron Salt	Route of Admin.	Modifying Factor	Doses (mg.Fe/g) and Mortality				LD_{50} mg.Fe/g.	95% Confidence Level of LD_{50} mg.Fe/g.
1. Ferrous Sulphate	I.V. rapidly	—	.008 0/5	.010 0/5	.013 3/5	.017 5/5	.013	.012 — .015
2. Ferrous Sulphate	I.V. slowly	—	.027 2/5	.033 4/5	.040 4/5	.049 5/5	.028	.023 — .035
3. Ferrous Sulphate	I.P.	—	.030 0/5	.042 2/5	.060 4/5	.055 5/5	.047	.038 — .058
4. Ferrous Sulphate	I.G.	—	.060 2/10	.121 3/10	.242 8/10	.483 8/10	.15	.141 — .16
5. Ferrous Sulphate	I.G.	Unstarved	.121 4/10	.181 4/10	.272 6/10	.408 6/10	.36	.25 — .51
6. Ferrous Sulphate	I.G.	$NaHCO_3$ I.P.	.121 0/5	.242 1/5	.484 2/5	.968 5/5	.45	.29 — .70
7. Ferrous Sulphate	I.G.	$NaHCO_3$ I.G.	.199 2/10	.282 2/10	.399 3/10	.564 7/10	46	.30 — .61
8. Ferrous Sulphate	I.G.	Desferal	.399 1/5	.564 0/10	.798 4/5	1.128 4/5	.75	.55 — 1.01
9. Ferrous Sulphate	I.G.	D.T.P.A.	.399 0/10	.564 2/10	.798 2/10	1.128 8/10	.86	.72 — 1.03
10. Ferric Chloride	I.V. Slowly	—	.016 0/5	.028 0/5	.050 3/5	.091 5/5	.049	.037 — .065
11. Ferric Chloride	I.G.	—	.186 1/10	.335 2/10	.604 8/10	1.087 10/10	.44	.30 — .63

I.V. = intravenous
I.P. = intra peritoneal
I.G. = intra gastric
D.T.P.A. = Diethylenetriaminepentacetate

Treatments to reduce the toxicity of the sulfate (and the chloride) were generally successful, using $NaHCO_3$, deferrioxamine, diethylenetriaminepentaacetate (149).

Rabbits

Nineteen female rabbits (about 5 lbs weight) were given intravenously doses of ferrous sulfate of from 50 to 80 mg/kg body weight. Electron microscope studies showed hepatacellular mitochondrial injury within four hours of injection; by eight hours considerable hepatic necrosis was evident and there were indications that iron is toxic for hepatocellular mitochondria. Mitochondrial damage may be the basis for the toxicity of acute iron overload (367).

Brown and Gray (42) following observations of a case of a small boy ingesting 40 ferrous sulfate tablets and recovering after intensive hospital therapy, performed an experiment in which ten rabbits (1.6-2.2 kg) were injected intravenously with 0.5 ml of ferrous sulfate solution containing 75 mg of the salt. Four of the test animals died within 24 hours; the remaining animals were sacrificed at 24 hours (1), 2 days (2), 3 days (1), 6 days (1) and 10 days (1). Brain, liver, spleen and kidneys were removed and prepared for histological examination. Estimates were made of plasma proteins and amino acid nitrogen. The minimum lethal dose appeared to be about 46 mg/kg. The reticulo-endothelial system was briefly saturated with iron; the liver necrotic; hypoglobulinemia evident and blood amino acid concentration was increased.

Pigs

Iron sulfate, lactate and glycerophosphate were orally administered in doses ranging from 450 to 600 mg/kg body weight to 8-day-old pigs. The sulfate and lactate produced behavioral changes, necroses in the gastric mucosa and distrophic changes in the liver. The glycerophosphate appeared to be harmless. The lactate produced emesis and some doses of the ferrous sulfate were lethal (248).

Dogs

Mongrel dogs of either sex weighing 6-14 kg were fed ferrous salts (carbonate, sulfate and gluconate) at various dose levels of the salt compressed into pellets and tablets of commercial preparations. The results of the initial experiment with seven dogs administered the chemical pellets are shown below:

Toxic effects in dogs of oral administration of ferrous salts

Dog	Body-weight (kg.)	Compound	Dose (g. ferrous iron per kg. body-weight)	General condition after administration of drug	Occurrence of			Post-mortem evidence of gastro-intestinal damage in						
					vomiting	malaise	death	fundus	pylorus	gastro-duodenal junction	duodenum	mid-intestine	ileum	large intestine
1	12·5	Ferrous carbonate	1·5	Normal	—	—	—	—	—	—	—	—	—	—
2	8·4	Ferrous carbonate	1·5	Normal	—	—	—	—	—	—	—	—	—	—
3	9·0	Ferrous carbonate	1·0	Normal	—	—	—	—	—	—	—	—	—	—
4	9·7	Ferrous sulphate	0·6	Severe diarrhœa present	+	+	+	+++	+++	+++	++	++	++	++
5	11·0	Ferrous sulphate	0·3	Diarrhœa present	+	—	—	++	++	+	±	—	—	—
6	10·5	Ferrous gluconate	0·75	Normal	+	—	—	+++	+++	+++	+	±	±	+
7	15·8	Ferrous gluconate	0·375	Normal	+	—	—	+	+	±	—	—	—	—

For vomiting, malaise or death, + = occurred. For post-mortem evidence of gastro-intestinal damage, + · · = severe ulceration; ++ = ulceration; + = inflammation, isolated areas of ulceration, or both; ± = slight inflammation; and — = no evidence of damage.

Additional studies were performed using the commercial tablets fed to more than fifty dogs. The tablets contained about 50 mg ferrous iron each of carbonate; sulfate, 60 mg; gluconate, 39 mg and succinate, 37 mg.

Examination of stomach tissues and intestine showed variable cellular necrosis and gastrointestinal pathology, most marked in ferrous sulfate treatment, somewhat less marked in gluconate and succinate, and least evident in the carbonate. Preferential use of the ferrous carbonate in treatment of iron deficiency anemia in children, particularly - is suggested (69).

The hemodynamic events, leading to shock following acute iron poisoning, were studied using 10 female mongrel dogs, administered intra-duodenally an LD_{100} dose of 225 mg/kg given as a 25% aqueous solution of ferrous sulfate (7 dogs); 3 dogs were dosed by gastric intubation. One hour after treatment, serum iron concentration increased; arterial pH decreased; hematocrit increased.. Other observations include: early marked reduction in cardiac output; progressive reduction in total blood volume; increased peripheral circulation hematocrit. The study suggests a need for restoration of effective blood volume in early therapy for iron poisoning cases in humans (362).

III. Long Term Studies

None

IV. Special Studies

Teratology

The influence of ferrous gluconate on the teratogenicity of salicylates was studied using Wistar rats as experimental animals. Sodium salicylate added to the diet of the rats has been shown to be an effective teratogen, as well as a chelating agent; abnormal embryos from rats fed salicylate lack otoliths. When the diet is supplemented by 2 mg of ferrous gluconate, alone - no abnormal embryos are observed: when supplementing the salicylate diet, a striking increase in resorptions and malformations occurs (173).

Pharmacology (therapy)

Hallberg et al. have made a study of the side-effects of various iron compounds used in iron-deficiency therapy. Some patients do not tolerate oral iron at dosages usually prescribed, which commonly range 150-300 mg elemental iron daily. Placebos and ferrous sulfate tablets were compared in one of several series of experiments. The subjects studied were 1496 blood donors who had not received previous iron supplements. Each subject received a bottle of tablets labelled "Iron tablets for blood donors" containing a two-week, 3 times a day supply. Bottles were coded and the iron tablets randomly distributed, the other subjects receiving pacebos. A group of 393 subjects received placebo tablets (195 subjects) and ferrous sulfate (198 subjects),

the daily dose being 222 mg iron. Of a total of 344 subjects who replied to a guestionnaire requesting information regarding side-effects, 13.6% had received a placebo and reported side-effects, 4.1% discontinuing treatment because of presumed reaction; of those receiving iron tablets, 22.9% reported side-effects with 8.0% discontinuing treatment. Side-effects reported were abdominal swelling, constipation, loose stools, and nausea (122).

Carcinogenecity

In a novel and somewhat unique report, Rommel states; "As a result of study and treatment I am of the opinion that cancer is an iron-deficiency disease and can be cured by the administration of iron, preferably ferrous sulfate." Five case reports of cancerous patients treated with ferrous sulfate are included. In his discussion, the author notes the presence of lowered hemoglobin as a general indication of tendency toward cancer and recommends the use of ferrous sulfate, particularly in early stages of cancer detection. "Then cancer can be prevented and in some cases cured." (291).

Biochemical Aspects

I. Breakdown

None

II. Absorption - Distribution

Kirksey et al. (176) have reported that iron intake of rats fed pyridoxine was approximately doubled by oral administration of $FeSO_4$ supplements containing 2 mg elemental iron daily during gestation. Two groups of 10 animals each of Sprague-Dawley female rats, 80 days of age, each received pyridoxine-deficient diets for 3 weeks prior to mating, and during gestation. Two other groups received 8 micrograms pyrodoxine/g of diet for the same period. One of each of the two groups throughout gestation received daily doses of 1 ml of a solution of $FeSO_4 \cdot 7H_2O$ in 1% HCl, equivalent to 2 micrograms of elemental iron. After 21 days of gestation the animals were sacrificed, placenta and fetus removed. Blood and tissues were analyzed for iron. Iron supplementation in the parent animal increased the total iron in maternal tissues, but the passage of iron from placenta to fetus was not increased. The mechanism preventing excessive transfer of iron to the fetus is discussed.

Fritz et al. (100) have studied the biological availability of various iron compounds from common dietary sources, with special attention to those that are, or might be used, for food fortification. Young chicks and rats were used as test animals, made anemic on a low-iron diet. Ferrous sulfate was used as a reference standard; the other compounds were added to the diet in quantities required to furnish the desired iron contribution to the diet. Hemoglobin and hematocrit determinations were made. Results using iron salts and ferrous sulfate as reference are shown below:

Comparison of Availability of Iron to Anemic Chicks and Anemic Rats

Iron Source	Relative Biological Values[a]	
	Chicks	Rats
Ferric ammonium citrate	115	98
Ferric orthophosphate #1	18	12
Ferric orthophosphate #2	9	12
Ferric orthophosphate #3	12	30
Ferric sulfate	65	100
Ferric oxide	4	6
Ferrous carbonate #1	2	1
Ferrous carbonate #2	2	0
Ferrous carbonate #3	6	0
Ferrous carbonate #4	2	2
Fish protein concentrate	22	53
Reduced iron #1	59	34
Reduced iron #2	41	16
Reduced iron #3	66	36
Reduced iron #4	43	37
Sodium iron pyrophosphate #1	2	11
Sodium iron pyrophosphate #2	13	19
Trace mineral mix (commercial)	14	21

[a] Relative biological value = (100 × mg Fe/kg from $FeSO_4$)/(mg Fe/kg from sample) to give equal curative effect.

Repletion tests were made on 21 iron compounds and on 14 food sources of iron. The values obtained with chicks and rats including data shown by species in the table above are shown in the following table:

Relative Biological Value of Iron from Various Dietary Sources

Iron Source	No. Samples	Relative Biological Value[a] Average	Range[b]
Iron Compounds			
EDTA, dihydrogen ferrous salt	1	99	97-100
Ferric ammonium citrate	1	107	98-115
Ferric choline citrate	1	102	
Ferric chloride	1	44	26-67
Ferric citrate	1	73	70-76
Ferric glycerophosphate	1	93	86-100
Ferric pyrophosphate	1	45	38-52
Ferric orthophosphate	4	14	7-32
Ferric oxide	1	4	0-6
Ferric sulfate	1	83	65-100
Ferrous ammonium sulfate	1	99	99-100
Ferrous carbonate	5	2	0-6
Ferrous chloride	1	98	
Ferrous fumarate	1	95	71-133
Ferrous gluconate	1	97	
Ferrous sulfate ($FeSO_4.7H_2O$)[a]	1	100	
Ferrous sulfate, anhydrous	1	100	
Ferrous sulfate, feed grade	1	100	
Ferrous tartrate	1	77	70-83
Reduced iron	6	37	8-66
Sodium iron pyrophosphate	3	14	2-23
Food and Feed Ingredients			
Biscuits with ferrous sulfate	1	89	77-100
Blood meal	1	35	
Corn meal enrichment mix[c]	1	46	
Corn germ	1	40	
Egg yolk	1	33	
Fish protein concentrate	2	28	8-53
Enriched breakfast cereal[c]	1	43	
Enriched flour[c]	1	32	
Oat flour	1	21	
Smectite-vermiculite	1	11	3-17
Soybean protein (isolated)	2	97	70-125
Trace mineral mix (commercial)[d]	2	12	0-21
Wheat germ	1	53	

[a] See footnote a, Table I. [b] Lowest and highest values are shown where more than one availability test was made. Note that this reflects variation both between samples and between repeated determinations on the same sample. [c] Fortified with reduced iron. [d] Fortified with ferrous carbonate.

In general, the results of these experiments support the view that inorganic iron compounds are better utilized than food iron.

The comparative iron retention of various iron compounds used for enrichment of bread and flour and the hemoglobin regeneration by anemic rats was studied by Freeman and Burrill (98). The rats used were distributed among the various groups studied by weight and sex in as

uniform a manner as possible. Anemia was produced by a milk diet until the hemoglobin concentration was about 3.0 gm/100 ml of blood. The iron compounds were administered both as salts mixed with cane sugar, and as supplemented bread prepared by the American Institute of Baking. The rats were killed after 28 days of supplemented diet, with hemoglobin determinations previously made on the 7th, 17th and 28th days. Carcasses were analyzed for iron content. The results are shown in the following table:

Iron retention and hemoglobin formation by anemic rats receiving iron supplements or iron enriched bread.

GROUP NO.	SOURCE OF Fe[1]	NO. RATS IN GROUP	AVG. PERIOD OF DEPLETION	AVG. WT. AT START OF SUPPLEMENT	AVG. WT. GAIN ON SUPPLEMENT	AVG. Hb AT START OF SUPPLEMENT	AVG. Hb INCREASE ON SUPPLEMENT		AVG. TOTAL Fe CONTENT OF CARCASS		RELATIVE Hb REGENERATION IN %	RELATIVE RETENTION OF Fe	% Fe SUPPLEMENT RETAINED
			days	gm.	gm.	gm./100 ml.	gm./100 ml.	C_r[2]	mg.	C_r[2]		%	
1	Ferric chloride	10	34	83	102	3.07	10.65		5.83				
2	Ferric chloride	10	36	89	107	2.96 3.01	9.72 10.00		5.60 5.75				
3	Ferric chloride	9	38	93	91	3.00	9.47 ± 1.17		5.82 ± 0.70		100	100	68.0
4	Sodium iron pyrophosphate	10	35	85	92	3.04	4.76 ± 1.74	2.5	3.19 ± 0.42	3.7	48.0	46.6	32.1
5	"Double amount" sodium iron pyrophosphate	6	36	90	55	2.97	6.18 ± 1.50	2.5	2.91 ± 0.13	3.6	62.3	40.9	14.0
6	Reduced iron	10	34	84	107	2.92	8.85 ± 0.89	0.8	5.15 ± 0.59	0.6	83.4	87.5	59.3
7	Sodium ferric orthophosphate	10	39	94	88	2.97	9.26 ± 1.52	0.4	5.26 ± 0.50	0.5	93.5	89.9	61.0
8	$FeCl_3$ bread	10	39	94	86	2.92	9.28 ± 1.10		5.61 ± 0.62		100	100	61.0
9	Sodium iron pyrophosphate bread	10	34	91	83	3.00	4.09 ± 1.10	3.5	3.32 ± 0.69	2.7	44.5	51.0	30.5
10	Reduced iron bread	9	44	91	93	2.78	7.95 ± 1.61	0.7	4.86 ± 1.00	0.5	86.0	84.0	51.0
11	Ferric orthophosphate bread	9	42	92	107	3.06	8.30 ± 1.48	0.5	5.27 ± 0.74	0.4	90.5	92.7	56.5
12	Sodium ferric orthophosphate bread	9	45	94	98	2.86	8.66 ± 1.13	0.5	5.29 ± 0.73	0.3	93.2	93.1	56.7
13	Plain bread	11	40	92	69	2.92	2.38 ± 1.09	4.9	2.33 ± 0.36	6.4	25.9	29.8	18.0

[1] Source of iron compounds (1, 2, 3, 8) Mallinckrodt, (4, 5, 9, 12) Victor Chemical, (6, 10, 11) Merck & Co.
[2] C_r = C.R. = critical ratio, considered significant when greater than 2 according to Fischer's rule.

The following order of biological effectiveness of the iron compounds studied resulted from the experiments: $FeCl_3$ was more effective than sodium ferric orthophosphate, is equal to ferric phosphate, is greater than ferrous Fe, is more effective than sodium iron pyrophosphate. No difference was observed as to whether the iron was given as the salt, or in the bread.

Hayasi (1939) performed a similar study, but in greater detail, regarding the body distribution of iron in rabbits. Mature, healthy Japanese rabbits were fed 0.6 g $FeCO_3$ daily for 2-35 days, sacrificed, and organs analyzed for iron content. Spleen, liver, bone marrow, appendix, colon, lung and kidney showed the greatest accumulation of iron (129).

An experiment similar to that of Freeman and Burrill was performed to test comparative biological availabilities of iron compounds in enriched bread by Blumberg and Arnold (33). Albino rats of the Sherman

strain were made anemic by an iron-depletion diet and fed bread containing various iron salts. The results are shown in the following table:

Responses of rats to various sources and levels of iron.

GROUP	BREAD: Compound added	BREAD: Iron added	NO. RATS	INITIAL WT. (AV.)	WT. GAIN (AV.): 1.5 wks.	WT. GAIN (AV.): 4 wks.	FOOD CONSUMPTION (AV.): 1.5 wks.	FOOD CONSUMPTION (AV.): 4 wks.	IRON INTAKE (AV.): 1.5 wks.	IRON INTAKE (AV.): 4 wks.	INITIAL HEMOGLOBIN (AV.)	HEMOGLOBIN GAIN MEAN ± S.E.: 1.5 wks.	HEMOGLOBIN GAIN MEAN ± S.E.: 4 wks.
		µg/gm		*gm*	*gm*	*gm*	*gm/day*	*gm/day*	*µg/day*	*µg/day*	*gm/100 ml*	*gm/100 ml*	*gm/100 ml*
1	None (negative control)		9	96	35	94	9.8	11.3	117	134	3.93	0.48 ± 0.26	3.69 ± 0.33
2	Ferric orthophosphate	8.4	8	84	36	90	9.4	12.3	177	231	3.90	0.69 ± 0.32	3.99 ± 0.44
3	Ferric orthophosphate	21.0	8	100	34	90	9.1	11.4	266	333	4.01	0.68 ± 0.33	5.64 ± 0.66
4	Ferric orthophosphate	52.5	9	92	45	111	10.5	12.9	576	708	3.86	2.96 ± 0.51	8.31 ± 0.57
5	Ferric orthophosphate	131.2	10	98	46	106	11.8	12.9	1389	1518	3.88	4.99 ± 0.76	11.11 ± 0.41
6	Ferrous sulfate	5.25	9	94	39	101	10.9	11.7	176	190	3.87	1.79 ± 0.14	5.84 ± 0.22
7	Ferrous sulfate	10.5	9	101	46	106	11.3	12.2	232	250	3.99	2.30 ± 0.37	7.82 ± 0.62
8	Ferrous sulfate	21.0	10	84	41	109	10.5	12.2	307	356	3.57	4.57 ± 0.39	9.48 ± 0.63
9	Ferrous sulfate	42.0	9	93	46	96	11.0	12.9	495	580	3.81	6.43 ± 0.28	11.62 ± 0.51
10	Ferric chloride	10.5	9	93	38	85	9.2	11.1	188	228	4.17	2.64 ± 0.45	8.62 ± 0.83
11	Ferric chloride	21.0	10	80	36	89	9.5	11.3	277	330	3.85	4.09 ± 0.29	10.77 ± 0.3
12	Ferrous sulfate (positive control)	244.0	7	84	45	101	8.7	11.1	1843	2352	3.39	9.23 ± 0.75	11.53 ± 1.8

While ferric sulfate and orthophosphate were studied, with the sulfate appearing to be 4 to 5 times available as the orthophosphate, the chief point is made in the observation that the ferric chloride was equal in biological activity to ferrous sulfate. Attention is drawn by the authors to the desirability of using highly assimilable forms of iron in flour and bread enrichment for maximum benefit to the consumer.

Moeller (229) studied absorption of iron compounds in piglets using radioactive Fe^{59} and showed that ferrous salts (ascorbate) were more readily absorbed than ferric salts (the ammonium citrate), and that the percentage absorbed was inversely related to the size of the dose. Ascorbic acid (0.25 and 0.5 gm) given before the iron dose increased its absorption, and daily ascorbic acid (0.5 and 1.0 gm) given before and during the experiment accelerated the incorporation of Fe into hemoglobin. Age of animal/absorption of iron was also studied, but no conclusions drawn.

Pyanovskaya et al. (275) have studied the effects of vegetable protein, hydrochloric acid and various dosages of iron and copper sulfates on productivity of fattened swine, noting also the level of deposited iron and copper in the animal's tissues. Five groups of pigs, each group uniform as to age and weight, were fattened from 70 days of age to attainment of 100 kg of live weight. All were fed a basic diet of cottonseed oil meal, barley, corn, wheat bran and green alfalfa.

Group I, the control, received a normal diet, generally utilized; Group II received a larger level of protein (cottonseed oil); Group III received additional trace elements in the iron and copper sulfates, plus an added 2 g of iron and 1 g copper sulfate; Group IV, same diet as III; Group V received a larger dosage of the sulfates. Group III also had added hydrochloric acid. After three months one swine from each group was sacrificed and an analysis of iron and copper in liver, thyroid gland, spleen and flesh was performed.

From 2 months to 4 months of age, Group III receiving large doses of both iron and copper and hydrochloric acid showed the greatest increase in weight. Both increased protein content and iron and copper are needed for optimal diet (fattening). The iron deposits were greatest in spleen and thyroid, least in the flesh.

Young dogs were fed raw whole milk supplemented with vitamins, Cu and Mn, resulting in anemia. The addition of 200-1000 gamma of Fe/kg body weight/day in the form of ferric pyrophosphate to the diet indicated that a minimum of 600 gamma of Fe was optimal in hemaglobin formation. Iron in excess of 600 gamma resulted in increased plasma Fe concentration; less than 600 gamma reduced plasma Fe below the critical 50 gamma of Fe/100 ml of plasma. Wheat bran and spinach were fed to supply 600 gamma of Fe/kg body weight/day in place of the pyrophosphate. The Fe in bran appeared almost completely available while the Fe in spinach was only 20-40% available (294).

Several forms of radioactive Fe^{59} were given orally to calves and sheep by Ammerman et al (10) to test the relative utilization of iron as influenced by the form in which it is given. Fe^{59} in ferric chloride, ferric carbonate, ferric oxide and ferrous sulfate was given in a single oral dose to calves and lambs. Fecal and urinary excretions were collected, as were blood samples, and the radioactivity measured with a liquid scintillation detector. Three separate experiments were performed: six dairy-type steer calves were studied in the first and second experiments, and 24 wethers in the last. Ranked on the basis of tissue Fe^{59} deposition, the sulfate was most utilized, followed in decreasing biological value by the carbonate, chloride and oxide, the latter significantly less effective. The special study of the chloride showed 3-5 fold more radioactivity from Fe^{59} than untreated calves.

Man

Ferrous chloride in the form of syrup and sugar-coated tablets was administered to 30 female patients of a Stockholm hospital, the doses of each form containing 0.15 g Fe^{++}. Initial normal serum iron concentration was first determined; blood samples were taken at 1, 2, 3 and 5 hours following the iron ingestion and serum iron concentration determined. The ferrous chloride syrup gave a rapid absorption and high serum iron values (249).

The absorption of iron as $FeSO_4$ in aqueous solution and tablets or in a plastic matrix is potentiated by ascorbic acid, as reported in a study by McCurdy and Dern (210). Male, prisoner volunteers were administered Fe^{55} or Fe^{59}-labeled preparation and the radioactivity of

blood samples was measured by liquid scintillation counter. The doses of ferrous sulfate varied from 15 to 120 mg; greater potentiation of ascorbic acid was at 500 mg of added acid. It is suggested that iron preparations containing ascorbic acid may permit less frequent doses in iron-deficiency therapy and may refill iron stores in the body better than iron salts without ascorbic acid.

Schulz and Smith (305) made a study of the influence of certain liquids and the size of the iron dose on the absorption of iron salts in normal and anemic infants and children. Ferrous sulfate was given both with tracer doses of radioactive Fe^{59} and as nonradioactive $FeSO_4$. One cubic centimeter of the dose contained 25 mg of iron. Thirty-four normal and five iron-deficient infants and children were studied; the larger single dose tolerated and absorbed well was 30 mg, of which 12% to 15% was absorbed by normal children when given once or twice a day. The addition of 180 cc milk or 100 cc orange juice to the iron salt decreased absorption of iron. Iron-deficient infants absorb more ferrous iron than do normal infants.

A method for making comparative studies of the absorbability of different iron compounds, utilizing two radioiron isotopes, Fe^{55} and Fe^{59} in ferrous and ferric sulfates is described. Alternate doses of the ferrous and ferric sulfates, of 5 and 20 mg content, were administered to 62 human subjects and blood samples analyzed for the iron isotopes by scintillation counter. The ferric iron salt used was $Fe_2(SO_4)_3 \cdot 6H_2O$. The absorbability of iron from ferrous and ferric sulfates was studied at different dosage levels and it was found that about 3-7 times more iron was absorbed from ferrous sulfate than from ferric sulfate (41).

The rate of gastrointestinal absorption of oral iron-dextran and ferrous sulfate was measured in 8 healthy subjects, 7 women and one man, by Ragen et al. (277). Known amounts of the iron compounds labelled with radioactive Fe^{59} were given in a single dose. The ferrous sulfate solution contained one microcurie of Fe^{59}/50 micrograms of elemental iron; the iron-dextran, and one microcurie Fe^{59}/354 micrograms of elemental iron. The stools of the patients were collected for 3 or 4 days, and the radioactivity measured with a scintillation counter. The range of absorption of the 50 microgram dose of ferrous sulfate was 29%-88%, an average of 52%; the iron-dextran dose of 354 micrograms ranged from 37% to 78%, an average of 51%.

Harrill (126) reports the effects of a low iron diet, using bread fortified with ferrous sulfate or ferric orthophosphate, in 9 young college women, over a period of 28 days. The iron content of food and feces was determined. The mean intake of iron during a control period was 5.43 mg; for the sulfate, 12.75 mg; and for the phosphate, 12.40 mg. Four percent of iron from ferrous sulfate bread, and three percent from the phosphate bread were absorbed by the women, on the average. Four subjects apparently absorbed none of the iron, and two absorbed large amounts. There was no significant change in hemoglobin values during the experiment. Larger amounts of the iron salts in flour enrichment would be of nutritional value.

Lapinleimu and Wegelius (193) studied the intestinal absorption of orally administered iron in infants and children with hypochronic anemia. The tests were performed on 18 children using ferric sodium ethylenediaminetetraacetate in doses of 152 mg, and ferrous gluconate, 132 mg. The serum iron values increased in both therapies in the same range. Additional tests of the therapeutic value of the iron chelate were performed on 402 children showing results comparable to the gluconate, but appearing more palatable, and not staining the teeth, as may appear in gluconate therapy.

Hoglund and Reizenstein (144) have performed several hundred absorption studies in 150 persons to determine the local intestinal factors with major roles in intestinal Fe absorption. The effect of iron dose and of ascorbic acid, food and iron therapy on radioiron Fe^{59} was studied. Normal iron absorption values were established in 24 male and 33 female volunteers; food effects were checked in 29 males and 4 females. All were healthy. Luminal iron concentration and ascorbic acid were studied in 25 healthy females; 26 males, healthy except for an iron deficiency were selected in studies of oral iron treatment and possible intracellular iron concentration increase in intestinal mucosa.

Four qualities of iron labelled with Fe^{59} were used: ferrous sulfate, ferrous fumarate and two metallic irons, one of "fine" particles and one of "course" particles of reduced iron. Reduced iron is customarily used to enrich flour in Sweden. Bread so enriched was used in the food studies as was the flour in "porridge".

Iron absorption in the various studies was measured using radioactive iron and a whole body counter. The diversity and scope of this study preclude details which may be seen in the papers following the bibliography of this monograph. The authors (144) summarize their findings as follows:

1. Since previous studies could not demonstrate that any of several general plasma factors played a major role in intestinal iron absorption, local intestinal factors were examined in 240 iron absorption studies on 150 healthy subjects.

2. When the iron dose was increased 40 times, from 0.25 to 10 mg, the percentage absorption was halved.

3. Trebling the quantity of food (bread) in the intestine did not significantly decrease absorption.

4. Ascorbic acid in the intestinal lumen trebled the absorption even of ferrous iron. A stable pharmaceutical combination of iron and ascorbic acid was tested.

5. Sifted flour did not seem to inhibit the absorption of ferrous iron, but coarse ground flour did. When fat was added, no further decrease in absorption was found although iron soaps may be formed.

6. A further decrease in absorption was found after a complete meal.

7. When fine grain reduced iron was used to enrich flour (this is done in all Swedish flour) absorption was 50 percent lower, and when a coarser grain reduced iron was used 85 percent lower, than when ferrous sulfate was used for enrichment.

8. When oral iron treatment was given to persons with high iron absorption, absorption was decreased to normal.

III. Metabolism and Excretion

Anemic chicks were fed a basal diet plus 0.1 mg Fe as $FeCl_3$, with and without the presence of small amounts of copper; the iron stimulates hemoglobin synthesis only in the presence of copper (84).

Anemic chicks were fed a basal diet plus 2 mg Fe in the form of Fe_2O_3, which proved ineffective due to lack of absorption of Fe_2O_3 by the chicks (84).

Four groups of adult male rats were given iron compounds by various routes: one group of 22 rats was given 0.6 mg iron as ferric ammonium citrate by gastric intubation. (The other groups are not relevant to this report). The animals were sacrificed at varying intervals from 2 1/2 to 6 hours after ingesting the iron. C^{14}-labelled leucine was injected intraperitoneally two hours before sacrifice. After killing, the intestine of each rat was removed, the mucosa scraped off and treated to separate the C^{14}-labelled ferritin whose activity was determined by a gas-flow Nuclear Chicago Counter. A three-fold increase over control animals in the synthesis of labelled ferritin in the intestinal mucosa occurred 4-5 hours after administration of the ferric ammonium citrate, falling to control levels at 6 hours. Ferritin protein remained, however, unchanged at the control level throughout the experiment (317).

Ghosh (109) studied the comparative biological availability of iron from ferrous sulfate, ferric chloride and ferric orthophosphate when used to fortify rice, which is a poor source of iron for hemopoiesis. Rats, six weeks old and anemic (hemoglobin level below 50%), ranging in body weight from 20 to 30 grams, were divided into 8 groups of six rats each. Two groups formed the controls, the remaining were fed iron-fortified rice diets, plus 0.03 mg of copper and weekly doses of 2 drops of Adexolin. The experimental feeding period lasted for 4 weeks; weekly hemoglobin determinations were made from blood samples. The results indicated that the ferric chloride and ferrous sulfate had a greater hemopoietic effect than the phosphate in enriched rice grain, with no appreciable difference between the chloride and the sulfate as hemopoietics.

One of the earliest studies encountered in the preparation of this monograph is by Bickel (28) who fed rats "Siderac", the magnetic ferrous-ferric oxide, and ferrous-ferric carbonate in a series of metabolism studies. The indeterminate nature of the iron compounds used permits only general conclusions. The iron diet apparently promotes growth in the animals, but too many variables render the studies of more historical interest than of scientific value.

The role of various crystalloidal and colloidal metallic compounds in nutritional anemia in rats was studied by Keil and Nelson (171). Rats were made anemic by milk diet and when the hemoglobin had fallen to 3.7 gm/100 cc, 0.50 mg of Fe as $FeCl_3$ and 0.05 mg Cu as $CuSO_4 \cdot 5H_2O$ were added to the basal diet. Eight weeks later the average hemoglobin (12 rats) was 402.5% of the anemic level. With 0.10 mg of manganese as $MnSO_4 \cdot 4H_2O$ replacing the copper, no regeneration of hemoglobin occurred. Iron as a colloid plus copper sulfate was effective, as was ferric chloride plus colloidal copper, in hemoglobin formation. Use of both iron and copper in colloidal form were effective. Zinc and magnesium similarly fed were ineffective. Copper as sulfide, hydroxide, oxide and iodide are all utilized in the production of hemoglobin.

The site of the action of the iron in acute intestinal iron poisoning is the subject of papers by Reissmann and Coleman (281) in which ferrous sulfate, gluconate and chloride were given to dogs and rabbits. This paper deals principally with the sulfate and with dogs. Following oral or rectal administration of the iron salts, rapidly and excessively absorbed iron produced profound metabolic acidosis with blood pH values as low as 6.7, due mainly to the hydrolysing effect of ferric ions and partly due to increase in lactic and citric acid, suggesting possible interference of iron with enzymes in the Krebs cycle.

Other changes, respiratory and circulatory, observed were hyperventilation, lowering of blood CO_2 and excessive CO_2 output, final respiratory failure followed by decrease in cardiac output and eventual failure, capillary congestion and increased permeability possibly due to high nonprotein bound serum iron, reduction in plasma volume and hemoconcentration. No abnormal hemoglobin derivatives were found.

Mehta et al. (213) studied the changes in serum iron following ingestion of 150 mg ferrous sulfate. Four groups of patients in a Bombay, India hospital were treated to observe the effects of anemia and succinic acid on iron absorption.

Group I: 30 patients, 10 anemic and 20 non-anemic. Blood samples collected after overnight fasting showed no significant variation in serum iron.

Group II: 65 patients, 35 anemic and 30 non-anemic. After collecting the first blood sample following overnight fasting, 150 mg ferrous sulfate powder were given and successive blood samples (2, 3 and 4 hours later) tested for serum iron. Maximum rise in serum iron was at 3 hours.

Group III: 75 patients, 45 anemic and 30 non-anemic. Blood samples were collected in the fasting state and 3 hours after ingestion of 150 mg ferrous sulfate. In the non-anemic patients the mean rise in serum iron was 81.8 micrograms/100 ml; in the anemic group the rise was 157.6 micrograms/100 ml.

Group IV: 20 patients all anemic were treated similarly to

Group III. Several days later studies were repeated with both the ferrous sulfate and succinic acid. The mean increase in serum iron was 80.9 micrograms.

Throughout the experiments a number of subjects showed rises in serum iron of less than 80.0 micrograms which was felt to be malabsorption of iron. Despite reports that average Indian diets contain ample iron supplies, the high incidence of non-deficiency anemia among Indian people suggests malabsorption of iron as a possible cause of the anemia.

A number of papers on the early uses (1927-28) of iron oxide (and iron carbonate) in metabolism and growth-promoting studies using rats, rabbits and dogs are presented here for their historical interest. Bickel (28) performed numerous feeding studies on rats, with results, while showing a positive effect of iron oxide on growth, were rather vague and not reproducible; the composition of the iron compounds used was not clearly understood. Goldbloom (114), a colleague of Bickel's, fed rabbits orally "active" and "inactive" iron oxides, counting red blood corpuscles and concluding that the treatment did not promote a better "blood picture" in normal, healthy rabbits. Additional experiments combining iron oxide and radiothorium treatments were performed, the Carbon/Nitrogen quotient of the urine determined. Results were unsatisfactory.

Rosenkranz (292), of the same laboratory, fed several dogs active iron oxide, determined urine nitrogen and carbon, and concluded that food was better utilized, nitrogen secretion in the urine decreased, protein oxidation decreased by 14-28%, the carbon secretion in the urine increased, and the body weight remained constant.

IV. Effects on Enzymes and Other Biochemical Parameters

A study of the metabolic interrelationships between calcium and iron was performed using young rats fed various diets. A diet of raw minced beef, supplemented by vitamins A and D, resulted in cessation of growth and indications of severe calcium deficiency. Adequate or excessive amounts of calcium carbonate restored appearance of good health. Diets of the meat either without calcium carbonate or an excess of carbonate developed anemia. Liberal additions of ferrous carbonate, regardless of calcium intake presented no signs of iron deficiency; stainable iron showed invariably in the spleen; the amount of iron appearing in the liver was inversely related to calcium intake and directly related to the iron intake (232).

Rehm and Winters (280) have studied the metabolic interrelationships of iron and the utilization of calcium and phosphorous in rats. Two groups of rats, six males and six females in each group, matched as to age, sex and weight were fed different diets. One group received a standard, artificial diet and the other the same diet supplemented by enough ferric chloride to combine with half the amount of phosphorous present in the diet. The animals were weighed at 4-day intervals; after 3 weeks two rats from each group were sacrificed and analyzed for total ash, calcium and phosphorous. At one month the experiment was

terminated. Despite careful control of food intake, the rats on the unsupplemented diet made greater gain weights than those receiving ferric chloride which appears to have a detrimental effect on calcium and phosphorous metabolism.

Forty New Zealand white female 2 kg rabbits were injected intravenously with a 25% aqueous solution of $FeSO_4 \cdot 7H_2O$. Single doses of 90 mg/kg produced some hepatic necrosis; additional injections of 50 and 90 mg at one and five hours after the first injection produced a higher incidence of hepatocellular injury. Four animals were sacrificed at 4 hrs and ten each at 8 and 12 hours following injection. Liver material was treated and the histochemistry performed to determine enzyme changes in treated animals. Initially, a number of oxidative enzymes and glucose-6-phosphatase increased in parenchymal cells, but then decreased. The disturbance of enzymes involved in the Krebs cycle; the citric and lactic acidemia observed in acute ferrous sulfate overload may serve as a biochemical basis for frequent unexplained death in acute ferrous sulfate intoxication in man (367).

Adult rhesus male monkeys (8.5 to 9.5 kg), were injected directly into the testes with ferrous sulfate and ferric chloride solutions (0.08 m-moles)/kg body weight). Two specimens were used for each of the two iron salts, and one control was injected with distilled water. One animal receiving each salt was sacrificed at 7 days, the other at 210 days. The results of the single injection showed an acute, irreversible degeneration of the testes affecting germinal and endocrine portions equally. The toxic properties of the ferrous and ferric salts act on the testes in a manner common to other heavy metal ions. The gonadotrophin content of the pituitary showed a consistent increase in the iron-treated animals (167).

Man

The response of 84 cases of hypochronic anemia in hospital patients to treatment by various forms and dosages of iron compounds includes the use of ferric ammonium citrate given orally. Blood samples taken, as a rule, every other day were analyzed for hemoglobin content. Each patient received individualized treatment with optimal doses of iron based on satisfactory rates of rise in hemoglobin during treatment. A dose of about one gram of iron was the maximum administered on a daily basis. The effects of iron therapy varied widely from one patient to another. Generally, these patients require prolonged treatment of months or indefinite duration (133).

Daily ingestion of 5 mg of iron supplement by infants during their first year (taken orally), beginning at one month of age produced a statistically significant increase in hemoglobin and hematocrit levels at three, six and nine months of age. This difference, however, at one year of age was not statistically significant. It was concluded that no real medical significance may be attached to this experiment (89).

Ferrous gluconate, vitamin B12 and ascorbic acid are mutually incompatible, but may be compounded into stable aqueous oral preparations using commercial 70% sorbitol solution as a vehicle (107).

V. Drug Interaction

A tablet containing ferrous sulfate in slow-release form combined with ascorbic acid was used in treatment of 45 patients, 34 of whom had straight-forward non-deficiency anemia, 5 had not responded to previous oral iron preparations, 5 had iron malabsorption and one woman with menorrhagia, awaiting an hysterectomy. 'Ferrograd C' combines ferrous sulfate 525 mg (equivalent to 105 mg ferrous iron) and ascorbic acid 500 mg as sodium ascorbate, held in a plastic matrix for slow release. Each patient received one tablet daily. Initial hemoglobin concentrations were determined and hemoglobin estimated at weekly intervals for one month.

In the 34 anemic patients without complications, the average daily rise in hemoglobin was 0.108 g/100 ml/day. Four of these patients had very low response, under 0.04 g/100 ml/day.

The five patients not previously responding to iron therapy had an average hemoglobin rise per day of 0.041 g/100 ml. Of the five malabsorption-of-iron patients, two responded to the combination of sulfate plus ascorbic acid.

The patient with menorrhagia increased her hemoglobin daily by 0.04 g/100 ml.

In general, the preparation tested with the ascorbic acid combined was marginally more effective than ferrous sulfate alone; the combination appeared more useful in those cases of iron malabsorption, however (155).

VII. Consumer Exposure Information

Iron, in its elemental form, as reduced or electrolytic iron, is used as a nutrient and/or dietary supplement, particularly by addition to flour. The various salts of iron are similarly widely used. The citrate (iron ammonium citrate) has been cleared for use as an anti-caking agent in table salt; ferrous gluconate, exempted from certification, has been listed for use in processing black olives; ferrous sulfate is most widely used in therapeutic doses is treatment of anemia. Many vitamin tablets include an iron salt as a preventative of anemia, but iron is present in most natural foods, particularly meat and eggs - and to a lesser degree in plant products.

The following tables were compiled from data submitted by user firms. Food consumption values for each food category were derived from the Market Research Corporation of America (MRCA) data on frequency of eating and from the USDA data on mean portion size of foods in each food category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of each substance.

Table 2 reports the usage of iron and iron salts and Table 3 their use in infant formulas or baby foods. Table 11 reports the annual poundage data for iron the various salts. Table 13 reports

the possible daily intake per food category and total dietary based on food consumption by total sample. Table 14 reports potential daily intakes in mg of NAS Appendix A substances (Groups 1 and 11) per food category reported, based on food consumption by eaters only.

TABLE 2 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
FERRIC PHOSPHATE NAS 0080	01	BAKED GOODS(R)	*	.00693	.01028
	02	BREAK CERLS(R)	6	.06385	.12875
	03	OTHER GRAIN(R)	5	.00763	.01258
	05	MILK PRODS(R)	5	.00614	.00886
	15	CONDM RELSH(R)	*	.00100	.00100
	23	BEV TYPE I(R)	*	.01602	.01602
	27	GRAVIES(R)	*	.01090	.01090
	28	IMIT DAIRY(R)	*	.01909	.01909
FERRIC PYROPHOSPHATE NAS 0081	02	BREAK CERLS(R)	*	.05000	.20000
	05	MILK PRODS(R)	*	.00273	.00273
	19	SWEET SAUCE(R)	*	.06000	.06000
	22	SNACK FOODS(R)	*	.00000	.00000
	23	BEV TYPE I(R)	*	.00000	.00000
	26	RECONST VEG(R)	*	.00620	.00620
FERRIC SODIUM PYROPHOS NAS 0082	01	BAKED GOODS(R)	*	.02098	.02098
	02	BREAK CERLS(R)	5	.14871	.50670
	03	OTHER GRAIN(R)	*	.01945	.02455
	05	MILK PRODS(R)	*	.02856	.02931
	10	MEAT PRODS(R)	*	.00160	.00160
	11	POULTRY(R)	*	.00900	.00900
	13	FISH PRODS(R)	*	.00900	.00900
	19	SWEET SAUCE(R)	*	.16300	.16300
FERROUS GLUCONATE NAS 0083	15	CONDM RELSH(R)	*	.00200	.00200
FERROUS SULFATE NAS 0085	01	BAKED GOODS(R)	9	.00441	.00696
	02	BREAK CERLS(R)	*	.01155	.10721
	03	OTHER GRAIN(R)	*	.00205	.02600
	05	MILK PRODS(R)	*	.00525	.03170
	10	MEAT PRODS(R)	*	.00106	.01060
	20	GELATIN PUD(R)	*	.01500	.01500
	23	BEV TYPE I(R)	*	.27500	.27500
	24	BEV TYPE II(R)	*	********	********
	34	INS COF TEA(R)	*	.00095	.09600

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TABLE II, PART A -- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1960	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
FERRIC PHOSPHATE NAS 0080	16/ 20	194,200	551,465	651,238			651,238
FERRIC PYROPHOSPHATE NAS 0081	6/ 6	850	1,506	1,506			1,506
FERRIC SODIUM PYROPHOS NAS 0082	11/ 12	404,933	401,983	402,880			402,880
FERROUS GLUCONATE NAS 0083	*/ *	4,000	2,200	2,200			2,200
FERROUS SULFATE NAS 0085	20/ 23	154,150	414,860	415,630			415,630
IRON, REDUCED NAS 0100	16/ 20	189,800	330,705	343,174			343,174

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 10/02/72

TABLE 3 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP II) USED IN INFANT FORMULA PRODUCTS & BABY FOODS(B)

NAS SURVEY NO.	SUBSTANCE NAME	FDDC CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
0082	FERRIC SODIUM PYROPHOS	83	FORMULAS(B)	*	.00534	.00557
0085	FERROUS SULFATE	83	FORMULAS(B)	8	.01034	.01307
0100	IRON, REDUCED	82	CEREALS(B)	*	.10000	.10000
		83	FORMULAS(B)	*	.01300	.01300

TABLE 13, PART A — POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
FERROUS SULFATE NAS 0085	02 BREAK CERLS(R)	*	0-5 MO.	.069300	.196350	.643260
			6-11 MO.	2.575650	6.906900	23.907830
			12-23 MO.	3.014550	5.878950	27.981810
			2-65+ YR.	2.310000	5.582900	21.442000
FERROUS SULFATE NAS 0085	03 OTHER GRAIN(R)	*	0-5 MO.	.010250	.034850	.130000
			6-11 MO.	.198850	.586300	2.522000
			12-23 MO.	.336200	.776950	4.264000
			2-65+ YR.	.569900	1.256700	7.228000
FERROUS SULFATE NAS 0085	05 MILK PRODS(R)	*	0-5 MO.	.283500	.210000	1.711800
			6-11 MO.	3.276000	15.755250	19.780500
			12-23 MO.	2.961250	9.156000	17.276500
			2-65+ YR.	2.073750	6.331500	12.521500
FERROUS SULFATE NAS 0085	10 MEAT PRODS(R)	*	0-5 MO.	.011660	.030740	.116600
			6-11 MO.	.219420	.591480	2.194200
			12-23 MO.	.320120	.550140	3.201200
			2-65+ YR.	.831040	1.379060	8.310400
FERROUS SULFATE NAS 0085	20 GELATIN,PUD(R)	*	0-5 MO.	.300000	.405000	.300000
			6-11 MO.	1.920000	5.820000	1.920000
			12-23 MO.	2.070000	5.040000	2.070000
			2-65+ YR.	3.060000	7.875000	3.060000
FERROUS SULFATE NAS 0085	23 BEV TYPE I(R)	*	0-5 MO.	6.600000	9.900000	6.600000
			6-11 MO.	62.425000	213.675000	62.425000
			12-23 MO.	149.050000	446.875000	149.050000
			2-65+ YR.	286.000000	763.675000	286.000000
FERROUS SULFATE NAS 0085	24 BEV TYPE II(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	**********	**********	**********
			12-23 MO.	**********	**********	**********
			2-65+ YR.	**********	**********	**********
FERROUS SULFATE NAS 0085	34 INS COF TEA(R)	*	0-5 MO.	.001900	.031350	.192000
			6-11 MO.	.050350	.124450	5.088000
			12-23 MO.	.058900	.212800	5.952000
			2-65+ YR.	1.150450	2.464300	116.256000
FERROUS SULFATE NAS 0085	83 FORMULAS(B)	8	0-5 MO.	34.711380	63.591000	43.875990
			6-11 MO.	7.072560	33.698060	8.939380
			12-23 MO.	2.274800	.641080	2.875400
FERROUS SULFATE NAS 0085	ALL CATEGORIES ********** ********** **********	23	0-5 MO.	42.137930	74.597740	53.806290
			6-11 MO.	78.857970	279.441820	128.545550
			12-23 MO.	162.389270	473.091100	216.464110
			2-65+ YR.	302.045660	797.954040	464.367020

TABLE 13, PART A — POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
FERRIC PHOSPHATE NAS 0080	01 BAKED GOODS(R)	*	0-5 MO.	.235620	.311850	.349520
			6-11 MO.	1.760220	3.589740	2.611120
			12-23 MO.	3.776850	6.223140	5.602600
			2-65+ YR.	9.507560	14.123340	14.104160
FERRIC PHOSPHATE NAS 0080	02 BREAK CERLS(R)	6	0-5 MO.	.383100	1.085450	.772500
			6-11 MO.	14.238550	38.182300	26.711250
			12-23 MO.	16.664850	32.499650	33.603750
			2-65+ YR.	12.770000	33.074300	25.750000
FERRIC PHOSPHATE NAS 0080	03 OTHER GRAIN(R)	5	0-5 MO.	.038150	.129710	.062900
			6-11 MO.	.740110	2.182130	1.220260
			12-23 MO.	1.251320	2.891770	2.063120
			2-65+ YR.	2.121140	4.684820	3.497240
FERRIC PHOSPHATE NAS 0080	05 MILK PRODS(R)	5	0-5 MO.	.331560	.245600	.478440
			6-11 MO.	3.831360	18.426140	5.528640
			12-23 MO.	3.346300	10.708160	4.828700
			2-65+ YR.	2.425300	7.404840	3.499700
FERRIC PHOSPHATE NAS 0080	15 CONDM RELSH(R)	*	0-5 MO.	**********	.001000	**********
			6-11 MO.	.008000	.022000	.008000
			12-23 MO.	.026000	.076000	.026000
			2-65+ YR.	.088000	.212000	.088000
FERRIC PHOSPHATE NAS 0080	23 BEV TYPE I(R)	*	0-5 MO.	.384480	.576720	.384480
			6-11 MO.	3.636540	12.447540	3.636540
			12-23 MO.	8.682840	26.032500	8.682840
			2-65+ YR.	16.660800	44.487540	16.660800
FERRIC PHOSPHATE NAS 0080	27 GRAVIES(R)	*	0-5 MO.	.010900	.032700	.010900
			6-11 MO.	.152600	.425100	.152600
			12-23 MO.	.392400	1.111800	.392400
			2-65+ YR.	.904700	2.321700	.904700
FERRIC PHOSPHATE NAS 0080	28 IMIT DAIRY(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	.267260	.439070	.267260
			12-23 MO.	.152720	.649060	.152720
			2-65+ YR.	.171810	.286350	.171810
FERRIC PHOSPHATE NAS 0080	ALL CATEGORIES ********** ********** **********	19	0-5 MO.	1.383810	2.383030	2.058740
			6-11 MO.	24.634640	75.714070	42.135670
			12-23 MO.	34.295280	80.192080	55.354130
			2-65+ YR.	44.649710	106.594890	64.676410
FERRIC PYROPHOSPHATE NAS 0081	02 BREAK CERLS(R)	*	0-5 MO.	.300000	.650000	1.200000
			6-11 MO.	11.150000	29.900000	44.600000
			12-23 MO.	13.050000	25.450000	52.200000
			2-65+ YR.	10.000000	25.900000	40.000000
FERRIC PYROPHOSPHATE NAS 0081	05 MILK PRODS(R)	*	0-5 MO.	.147420	.109200	.147420
			6-11 MO.	1.703520	8.192730	1.703520
			12-23 MO.	1.487850	4.761120	1.487850
			2-65+ YR.	1.078350	3.292380	1.078350

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	POSSIBLE DAILY INTAKE, MG. (AGE)	AVERAGE	HIGH A	HIGH B
FERRIC PYROPHOSPHATE NAS 0081	19 SWEET SAUCE(R)	*	0-5 MO.	.180000	.240000	.180000
			6-11 MO.	.540000	1.860000	.540000
			12-23 MO.	1.560000	4.560000	1.560000
			2-65+ YR.	4.080000	10.740000	4.080000
FERRIC PYROPHOSPHATE NAS 0081	22 SNACK FOODS(R)	*	0-5 MO.	**********	.000000	**********
			6-11 MO.	.000000	.000000	.000000
			12-23 MO.	.000000	.000000	.000000
			2-65+ YR.	.000000	.000000	.000000
FERRIC PYROPHOSPHATE NAS 0081	23 BEV TYPE I(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	.000000	.000000	.000000
			12-23 MO.	.000000	.000000	.000000
			2-65+ YR.	.000000	.000000	.000000
FERRIC PYROPHOSPHATE NAS 0081	26 RECONST VEG(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	.000000	.000000	.000000
			12-23 MO.	.000000	.000000	.000000
			2-65+ YR.	.012400	.037200	.012400
FERRIC PYROPHOSPHATE NAS 0081	ALL CATEGORIES ********** ********** **********	6	0-5 MO.	.627420	1.199200	1.527420
			6-11 MO.	13.393520	39.952730	46.843520
			12-23 MO.	16.097850	34.771120	55.247850
			2-65+ YR.	15.170750	39.969580	45.170750
FERRIC SODIUM PYROPHOS NAS 0082	01 BAKED GOODS(R)	*	0-5 MO.	.713320	.944100	.713320
			6-11 MO.	5.328920	10.867640	5.328920
			12-23 MO.	11.434100	18.840040	11.434100
			2-65+ YR.	26.784560	42.757240	28.784560
FERRIC SODIUM PYROPHOS NAS 0082	02 BREAK CERLS(R)	5	0-5 MO.	.892260	2.528070	3.040200
			6-11 MO.	33.162330	88.928580	112.994100
			12-23 MO.	38.813310	75.693390	132.248700
			2-65+ YR.	29.742000	77.031780	101.340000
FERRIC SODIUM PYROPHOS NAS 0082	03 OTHER GRAIN(R)	*	0-5 MO.	.097250	.330650	.122750
			6-11 MO.	1.886650	5.562700	2.391350
			12-23 MO.	3.168800	7.371550	4.026200
			2-65+ YR.	5.407100	11.942300	6.824900
FERRIC SODIUM PYROPHOS NAS 0082	05 MILK PRODS(R)	*	0-5 MO.	1.542240	1.142400	1.582740
			6-11 MO.	17.821440	85.708560	18.299440
			12-23 MO.	15.565200	49.808640	15.973950
			2-65+ YR.	11.281200	34.443360	11.577450

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	POSSIBLE DAILY INTAKE, MG. (AGE)	AVERAGE	HIGH A	HIGH B
FERRIC SODIUM PYROPHOS NAS 0082	10 MEAT PRODS(R)	*	0-5 MO.	.017600	.046400	.017600
			6-11 MO.	.331200	.892800	.331200
			12-23 MO.	.483200	.830400	.483200
			2-65+ YR.	1.254400	2.081600	1.254400
FERRIC SODIUM PYROPHOS NAS 0082	11 POULTRY(R)	*	0-5 MO.	.045000	.207000	.045000
			6-11 MO.	.351000	1.188000	.351000
			12-23 MO.	.594000	1.656000	.594000
			2-65+ YR.	1.161000	2.952000	1.161000
FERRIC SODIUM PYROPHOS NAS 0082	13 FISH PRODS(R)	*	0-5 MO.	.009000	.027000	.009000
			6-11 MO.	.117000	.441000	.117000
			12-23 MO.	.486000	1.215000	.486000
			2-65+ YR.	1.116000	2.781000	1.116000
FERRIC SODIUM PYROPHOS NAS 0082	19 SWEET SAUCE(R)	*	0-5 MO.	.489000	.552000	.489000
			6-11 MO.	1.467000	5.053000	1.467000
			12-23 MO.	4.238000	12.369000	4.238000
			2-65+ YR.	11.084000	29.177000	11.084000
FERRIC SODIUM PYROPHOS NAS 0082	83 FORMULAS(B)	*	0-5 MO.	17.926380	32.841000	18.698490
			6-11 MO.	3.652560	17.403060	3.809880
			12-23 MO.	1.174800	.331000	1.225400
FERRIC SODIUM PYROPHOS NAS 0082	ALL CATEGORIES	14	0-5 MO.	21.732050	38.718620	24.718100
	**********		6-11 MO.	64.118100	216.045340	145.068290
	**********		12-23 MO.	75.978410	168.134100	170.709550
	**********		2-65+ YR.	89.830260	203.166280	163.142310
FERROUS GLUCONATE NAS 0083	15 CONDM RELSH(R)	*	0-5 MO.	**********	.002000	**********
			6-11 MO.	.016000	.044000	.016000
			12-23 MO.	.056000	.152000	.056000
			2-65+ YR.	.176000	.424000	.176000
FERROUS GLUCONATE NAS 0083	ALL CATEGORIES	*	0-5 MO.	**********	.002000	**********
	**********		6-11 MO.	.016000	.044000	.016000
	**********		12-23 MO.	.056000	.152000	.056000
	**********		2-65+ YR.	.176000	.424000	.176000
FERROUS SULFATE NAS 0085	01 BAKED GOODS(R)	9	0-5 MO.	.149940	.199450	.236640
			6-11 MO.	1.120140	2.284300	1.767840
			12-23 MO.	2.403450	3.960180	3.793200
			2-65+ YR.	6.050520	8.987580	9.549120

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Condm Relish	Ferrous gluconate	*	0-5 mo	.008	.012	.008	.012
			6-11 mo	.036	.068	.036	.068
			12-23 mo	.084	.194	.084	.194
			2-65 yr	.218	.458	.218	.458
Baked Goods	Ferric phosphate	*	0-5 mo	1.074	3.555	1.593	5.274
			6-11 mo	1.989	3.777	2.950	5.603
			12-23 mo	3.805	6.223	5.644	9.231
			2-65 yr	9.515	14.130	14.114	20.961
Break Cerls	Ferric phosphate	6	0-5 mo	3.256	8.492	6.566	17.124
			6-11 mo	21.134	41.566	42.616	83.816
			12-23 mo	18.133	32.819	36.565	66.178
			2-65 yr	17.112	35.437	34.505	71.456
Other Grain	Ferric phosphate	5	0-5 mo	.679	1.419	1.120	2.340
			6-11 mo	1.381	3.166	2.277	5.221
			12-23 mo	1.518	3.029	2.503	4.994
			2-65 yr	2.609	5.135	4.302	8.466
Condm Relish	Ferric phosphate	*	0-5 mo	.004	.006	.004	.006
			6-11 mo	.018	.034	.018	.034
			12-23 mo	.042	.097	.042	.097
			2-65 yr	.109	.229	.109	.229
Bev Type I	Ferric phosphate	*	0-5 mo	5.223	21.691	5.223	21.691
			6-11 mo	7.545	16.885	7.545	16.885
			12-23 mo	13.008	29.909	13.008	29.909
			2-65 yr	22.796	51.088	22.796	51.088
Gravies	Ferric phosphate	*	0-5 mo	.120	.229	.120	.229
			6-11 mo	.338	.643	.338	.643
			12-23 mo	.763	1.526	.763	1.526
			2-65 yr	1.450	2.878	1.450	2.878
Imit Dairy	Ferric phosphate	*	0-5 mo	.000	.000	.000	.000
			6-11 mo	3.169	3.990	3.169	3.990
			12-23 mo	1.584	2.864	1.584	2.864
			2-65 yr	1.107	3.245	1.107	3.245

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Break Cerls	Ferric pyrophosphate	*	0-5 mo	2.550	6.650	10.200	26.600
			6-11 mo	16.550	32.550	66.200	130.200
			12-23 mo	14.200	25.700	56.800	102.800
			2-65 yr	13.400	27.750	53.600	111.000
Reconst Veg	Ferric pyrophosphate	*	0-5 mo	---	---	---	---
			6-11 mo	---	---	---	---
			12-23 mo	---	---	---	---
			2-65 yr	.112	.186	.112	.186
Baked Goods	Ferric sodium pyrophos	*	0-5 mo	3.252	10.763	3.252	10.763
			6-11 mo	6.021	11.434	6.021	11.434
			12-23 mo	11.518	18.840	11.518	18.840
			2-65 yr	28.806	42.778	28.806	42.778
Break Cerls	Ferric sodium pyrophos	5	0-5 mo	7.584	19.778	25.842	67.391
			6-11 mo	49.223	96.810	167.718	329.862
			12-23 mo	42.234	76.437	143.903	260.444
			2-65 yr	39.854	82.534	135.796	281.219
Meat Prods	Ferric sodium pyrophos	*	0-5 mo	.174	.437	.174	.437
			6-11 mo	.482	.979	.482	.979
			12-23 mo	.507	.840	.507	.840
			2-65 yr	1.267	2.086	1.267	2.086
Formulas	Ferric sodium pyrophos	*	0-5 mo	21.515	34.860	22.442	36.361
			6-11 mo	15.598	32.558	16.270	33.960
			12-23 mo	19.155	39.714	19.980	41.424
			2-65 yr	---	---	---	---
Baked Goods	Ferrous sulfate	9	0-5 mo	.684	2.262	1.079	3.570
			6-11 mo	1.266	2.403	1.998	3.793
			12-23 mo	2.421	3.960	3.821	6.250
			2-65 yr	6.055	8.992	9.556	14.191
Break Cerls	Ferrous sulfate	*	0-5 mo	.589	1.536	5.468	14.259
			6-11 mo	3.823	7.519	35.487	69.794
			12-23 mo	3.280	5.937	30.448	55.106
			2-65 yr	3.095	6.410	28.732	59.502

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Meat Prods	Ferrous sulfate	*	0-5 mo	.116	.289	1.155	2.894
			6-11 mo	.319	.649	3.191	6.487
			12-23 mo	.336	.557	3.360	5.565
			2-65 yr	.840	1.382	8.395	13.822
Gelatin Pud	Ferrous sulfate	*	0-5 mo	3.525	10.830	3.525	10.830
			6-11 mo	3.435	7.170	3.435	7.170
			12-23 mo	3.015	6.225	3.015	6.225
			2-65 yr	4.635	9.390	4.635	9.390
Ins Caf Tea	Ferrous sulfate	*	0-5 mo	.027	.132	2.688	13.344
			6-11 mo	.154	.396	15.552	40.032
			12-23 mo	.134	.346	13.536	34.944
			2-65 yr	1.360	2.529	137.376	255.552
Formulas	Ferrous sulfate	8	0-5 mo	41.660	67.500	52.659	85.321
			6-11 mo	30.203	36.043	38.178	79.688
			12-23 mo	37.090	76.899	46.882	97.202
			2-65 yr	---	---	---	---
Baked Goods	Iron, reduced	14	0-5 mo	.388	1.283	.642	2.124
			6-11 mo	.718	1.363	1.188	2.256
			12-23 mo	1.373	2.245	2.273	3.718
			2-65 yr	3.433	5.098	5.684	8.441
Break Cerls	Iron, reduced	8	0-5 mo	.874	2.280	2.050	5.347
			6-11 mo	5.673	11.158	13.306	26.170
			12-23 mo	4.868	8.810	11.417	20.663
			2-65 yr	4.594	9.513	10.774	22.311
Other Grain	Iron, reduced	8	0-5 mo	.264	.552	.347	.725
			6-11 mo	.538	1.233	.706	1.619
			12-23 mo	.591	1.179	.776	1.548
			2-65 yr	1.016	1.999	1.334	2.625
Cereals	Iron, reduced	*	0-5 mo	15.300	23.500	15.300	23.500
			6-11 mo	15.000	30.500	15.000	30.500
			12-23 mo	12.000	18.200	12.000	18.200
			2-65 yr	---	---	---	---

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Formulas	Iron, reduced	*	0-5 mo	52.377	84.864	52.377	84.864
			6-11 mo	37.973	79.261	37.973	79.261
			12-23 mo	46.631	96.681	46.631	96.681
			2-65 yr	---	---	---	---

TABLE 15

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Bev Type I	Ferric chloride	*	0-5 mo	.522	2.166	.522	2.166
			6-11 mo	.754	1.686	.754	1.686
			12-23 mo	1.299	2.987	1.299	2.987
			2-65 yr	2.277	5.102	2.277	5.102

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Robert A. Aldrich, M.D.

ACUTE IRON TOXICITY

Before the past decade, acute toxicity from ingestion of therapeutic iron preparations was infrequently reported in the medical literature. In 1947, Forbes [1] drew attention to the occurrence of accidental poisoning of small children by ferrous sulfate in the oral medications being prescribed for adults. Subsequently there have been many reported cases of iron poisoning, usually by iron sulfate tablets not intended for children. Since the mortality rate from acute iron poisoning is approximately 50 per cent, this topic warrants careful consideration.

CASE REPORTS OF ACUTE IRON TOXICITY IN CHILDREN

The clinical features, epidemiology, pathology, treatment, and prevention of iron poisoning in children have been studied in 42 case reports from the medical literature [1–23].

Clinical Features

The essential ingredients that lead to acute iron toxicity are, first, a curious child who is able to walk and, possibly, climb and, second, a box or bottle of ferrous sulfate tablets. The tablets are generally found on a table, shelf, or in a drawer. The toddler apparently finds it difficult to resist the lure of tablets that look like candy, and in eating them he may be trying to emulate his mother, whom he has observed taking the tablets. Most children will play for a moment with the brightly colored tablets, but others may begin eating them almost immediately, especially if they have seen others taking them. If the tablets are covered with chocolate or colored sugar coating, this material may be found on the hands, clothes, or face—thus providing the parent with

a clue to the type of poison. If the tablets have been well chewed, there may be pieces of the tablets available for analysis.

The acute iron toxicity syndrome can be divided into four chronological phases.

First phase.—The first symptoms of acute iron poisoning begin approximately 30–40 minutes after eating the tablets. The child complains of pain or an uncomfortable feeling in the abdomen, and soon vomits. The vomits often contains a few of the tablets, either fragmented or intact, depending on how much they have been chewed. The color of the stomach contents is brown, but blood may be present. Very soon the patient becomes irritable and pale, drowsiness develops, and the pulse weakens. Classical signs and symptoms of cardiovascular collapse intervene, and it is generally at this stage that hospitalization occurs. Accompanying this part of the illness, there is nearly always a diarrhea that is characterized by stools of green or black color and watery consistency. Respirations have been described in some cases as resembling the "air hunger" of diabetic acidosis. It is in this first phase that approximately one-fifth of all children poisoned with iron expire. The cardiovascular collapse becomes more profound, and coma leads to death in less than 6 hours.

Second phase.—If the patient can be sustained throughout these first few hours there is almost always some improvement noted, with color, pulse, respirations, and consciousness approaching normal. The child often awakens from his stupor and recognizes his family and surroundings. Vomiting and diarrhea diminish in severity, and the physician may become unduly optimistic. This improvement lasts approximately 10–14 hours more. Then one of two trends develops: either the child continues to improve and recovers or he suddenly relapses.

Third phase.—Relapse from the course of progressive improvement is marked by a sudden return to severe and usually irreversible cardiovascular collapse. Shock becomes profound, respirations change to Cheyne-Stokes type, and convulsions are followed by coma and death. This part of the syndrome takes place approximately 20 hours after the ingestion of the iron.

Fourth phase.—There is a very late manifestation of this clinical condition evident in those who recover. Between the end of the first month and the latter part of the second month, gastric obstructive signs and symptoms from scarring of the stomach appear. Late cicatricial change occurred in the stomachs of 5 children in this series. Four of the patients

underwent successful surgical repair, but the fifth died from malnutrition and never was in condition for operative intervention.

Epidemiology: Consideration of Data in Table 1

The age incidence among these 42 children seems to be by far the most significant epidemiologic statistic in table 1. The youngest was an 11-month-old infant and the oldest was a 4½-year-old preschool child. However, 34 of the 42 individuals in this group were between the ages of 12 and 24 months inclusive, a fact that illustrates clearly where four-fifths of the affected population can be located by age. It is of incidental interest that a 26-year-old man was fatally poisoned with ferrous sulfate, but this was not a therapeutic preparation [24]. There was no significant difference between males and females in the incidence of iron toxicity, but this may be due to the limited age range affected.

With one exception, the children in this series were poisoned by ferrous sulfate, and all except two received the iron in tablet form. Dosages varied greatly, and this range might have been wider if true dosages could be established in all cases. It is notable from inspection of the table that there must be other factors that determine susceptibility to the toxic effects of iron, because as little as 4.8 gm. resulted in death on one occasion [20], but recovery followed ingestion of the relatively huge dose of 15 gm. [16, 20]. The range of dosage in the fatal poisonings was 4.8–18.0 gm., whereas for nonfatal cases it was 1.5–15.0 gm.

Therapeutic measures were chiefly distinguished by their multiplicity and relative ineffectiveness. However, it is instructive to read the original reports of these cases and follow the rationale of each form of treatment. Shocklike symptoms accompanying cardiovascular collapse were combatted with plasma, whole blood, or other intravenous fluids, usually normal saline. Oxygen was administered to several children in an effort to overcome poor color and respiratory distress. Systemic therapy with B.A.L. was attempted in an effort to inactivate the large amounts of excess iron. Local measures to remove iron from the stomach and gastrointestinal tract included lavage with sodium bicarbonate to convert the iron to the more insoluble carbonate form. Magnesium sulfate and bismuth subcarbonate were also used in lavage fluids. In a few cases cathartics were administered to clear the gastrointestinal tract of the offending substance. A vitamin and amino-acid formula devised by Spencer was used in the treatment of some patients [8, 20]. The most effective measures seemed to be those directed toward overcoming shock during the first phase of the poisoning and removing the remaining iron from the stomach and gastrointestinal tract.

The duration of the illness after ferrous sulfate ingestion was related, of course, to whether or not the outcome was fatal. Eight of the patients died in less than 6 hours. Thus 42 per cent of the fatalities and 19 per cent of the patients in the group succumbed in the first phase. These deaths were secondary to cardiovascular collapse and irreversible shock. Another 9 children expired between 20 and 53 hours after ingesting the iron. This time interval corresponds to the third phase, during which 47 per cent of the fatalities occurred. Thus approximately 90 per cent of all deaths from iron poisoning in children occur during these two distinct clinical phases of this syndrome. The 2 patients that were not in these groups died at 11 hours and at 16 weeks, the former from cardiovascular shock and the latter from profound malnutrition secondary to gastric stricture. There were 5 children who survived the late complication of scarring and contracture of the stomach following appropriate surgical intervention between the end of the first month of their illness and the latter part of the second month. The operative results seem to have been satisfactory.

For the total group of 42 children there was a mortality rate of 45 per cent, but if one adds to this the 5 patients who underwent major surgery because of scarring of the stomach and duodenum, the outlook for a recovery without residue from acute iron poisoning becomes even more limited. The final figure on both fatalities and sequelae requiring major surgery is 57 per cent of all affected patients.

Pathologic Manifestations of Acute Iron Toxicity

Severe necrotizing gastritis occurs in virtually every patient. The mucosal surfaces are hemorrhagic and extensive sloughing is present. If the ingested iron is in the form of enteric coated tablets, segments of the small bowel develop a severe necrotizing process at various levels of the gastrointestinal tract, depending on the site at which the ferrous sulfate is liberated from the tablet. If enteric coated tablets reach the jejunal parts without releasing their contents, they probably have not been chewed. The extensive tissue changes are the result of high concentrations of the ions—ferric, sulfate, chloride, and hydrogen—formed in the gastric juice. The presence of a strongly acid solution containing heavy metal ions is likely to produce coagulation of protein and extensive corrosion. Microscopically it is seen that the lesions in the gastric mucosa are mainly on the tips of the rugae and represent superficial necrosis and ulceration. The stomach mucosa is diffusely congested and infiltrated with polymorphonuclear leucocytes and mononuclear cells. Platelet thrombi are numerous in the submucosal capillaries and veins, and special stains demonstrate both ferrous and ferric iron in the super-

TABLE 1

ACUTE IRON TOXICITY

Age (months)	Iron	Dosage taken	Treatment	Duration of illness	Outcome	Ref.
39	$FeSO_4$ tabs.	10 gm.	none	53 hours	fatal	
12	$FeSO_4$ tabs.	6 gm.	supportive	30 hours	fatal	[1]
13	$FeSO_4$ tabs.	6 gm	oxygen, lavage, transfusion	dismissed from hospital 8 days	recovered	[2]
16	$FeSO_4$ tabs.	5 gm.	lavage $NaHCO_3$	21 hours	fatal	
24	$FeSO_4$ tabs.	1.6 gm.	supportive	dismissed from hospital 14 days	recovered	[3]
15	$FeSO_4$ tabs.	2.6 gm.	supportive	dismissed 4 days	recovered	
20	$FeSO_4$ tabs.	16 gm.	O_2, transfuse	21 hours	fatal	[4]
21	$FeSO_4$ caps.	"large number"	transfuse	48 hours	fatal	[5]
18	$FeSO_4$ tabs.	"unknown"	transfuse	dismissed 11 days	recovered	[6]
19	$FeSO_4$ tabs.	2.0 gm.	lavage and supportive	36 hours	recovered	[7]
26	$FeSO_4$ caps.	13 gm.	lavage, Spencer formula, BAL, transfuse	dismissed 6 days	recovered	[8]
15	$FeSO_4$ tabs.	8.6 gm.	O_2 lavage	4 hrs. 15 min.	fatal	[9]
21	$FeSO_4$ tabs.	"unknown"	lavage	surgery in 5 weeks	recovered after surgical repair of scar	[10]
24	$FeSO_4$ tabs.	8.0 gm.	lavage $NaHCO_3$	surgery in 5½ weeks		
54	$FeSO_4$ tabs.	7.2 gm.	vomiting, bismuth subcarb.	"short"	recovered	
19	$FeSO_4$ tabs.	3.0 gm.	lavage with $NaHCO_3$ BAL.	48 hours	recovered	[11]
30	$FeSO_4$ tabs.	4.5 gm.	lavage $MgSO_4$	dismissed 3 days	recovered	
29	$FeSO_4$ tabs.	"unknown" (?) 18 gm.	transfusion, supportive	4½ hours	fatal	[12]
15	$FeSO_4$ tabs.	6.0 gm.	supportive	48 hours	recovered	
18	$FeSO_4$ tabs.	4.5 gm.	plasma, lav.	dismissed 5 days	recovered	[13]
26	$FeSO_4$ tabs.	"unknown"	O_2 lavage	4½ hours	fatal	
19	$FeSO_4$ tabs.	"unknown"	transfuse BAL, support.	40 hours	fatal	[14]
17	$FeSO_4$ tabs.	(?) 3 gm.	transfuse	acute illness for 13 days, surgery on 58th day	pyloric stenosis recovered after surgery	[15]
13	$FeSO_4$ tabs.	"unknown"	plasma, lavage $NaHCO_3$	acute–19 days, surgery 45th day.	recovery, postop.	
30	$FeSO_4$ tabs.	15.0 gm.	saline lavage	dismissed 11 days	recovered	[16]
17	$FeSO_4$ tabs.	6.0 gm.	plasma, methylene blue, supportive	11 hours	fatal	[17]

ficial mucous membrane, connective tissue, basement membrane, and endothelial lining of these vessels and the lymphatics [9, 19, 23].

Pyloric, duodenal, and small bowel lesions farther along the gastrointestinal tract have essentially the same pathology. The lower part of the ileum and the colon may show ferric and ferrous iron on the mucous membrane suface, in platelet thrombi, and in the subserosal small vessels.

The contents of the entire intestinal lumen have usually been described as blackish-green or gray, sometimes bloody, material. Mesenteric adenitis and thrombosis of the mesenteric veins have also been described [14].

The liver is also altered by acute iron toxicity. There have been gross reports of swelling, a "nutmeg" appearance, and small hemorrhagic areas [19]. Microscopically there is cloudy swelling and hemorrhagic periportal necrosis. Iron stains of these affected areas show finely dispersed iron deposits in portal vein endothelium, Kupffer cells, and periportal reticulum. Published descriptions of changes in the liver tissues visible under the light microscope do not seem sufficiently specific to explain the mechanism of death.

The lungs, brain, and kidneys also show edema, cloudy swelling, and areas of hemorrhage. These nonspecific changes are rather widespread in all of the viscera, including the heart. It is of interest that pathologists have commented on the flabby state of the myocardium, especially the right atrium and ventricle [9, 23].

Noncoagulability of the blood at the time of post-morten examination has been noted, although there have been no reports of coagulation studies among survivors of acute iron poisoning [4, 9].

Clinical chemical investigations of individuals suffering from acute iron toxicity have demonstrated very high plasma iron levels, marked leucocytosis, increased serum bilirubin values, and low plasma bicarbonate. There has been no convincing explanation of any but the first of these findings.

Experimental Studies and the Pathologic Physiology of Acute Iron Toxicity

There seems to be no doubt that iron was responsible for the toxic effects observed by Forbes [1]. The pills that he tested on cats and guinea pigs contained manganese and copper sulfates as well as ferrous sulfate. He was able to show that the copper and manganese salts were without toxic effect, but that the iron salt produced toxic results very similar to those he had observed clinically in children who had accidentally received large amounts of iron.

TABLE 1—*Continued*

Age (months)	Iron	Dosage taken	Treatment	Duration of illness	Outcome	Ref.
16	$FeSO_4$ tabs.	"unknown"	transfusion	12 days acute illness, 60 days until surgery	recovered following partial gastrectomy for pyloric stenosis	[18]
16	$FeSO_4$ tabs.	"unknown"	supportive	31 hours	fatal	
18	$FeSO_4$ tabs.	"unknown"	lavage, supportive	20 hours	fatal	[19]
12	$FeSO_4$ tabs.	"unknown"	supportive	4 hours	fatal	
14	$FeSO_4$ tabs.	15–20 gm.	BAL, lavage, fluid	dismissed 9 days	recovered	[20]
21	$FeSO_4$ tabs.	15.0 gm.	lavage $NaHCO_3$	dismissed 11 days	recovered, mental signs only while sick	
23	$FeSO_4$ tabs.	6.5 gm.	supportive	dismissed 6 days	recovered	
11	$FeSO_4$ tabs.	3.9 gm.	lavage	dismissed 3 days	recovered	
20	$FeSO_4$ tabs.	1.5 gm.	none	dismissed 24 hours	recovered	
12	$FeSO_4$ tabs.	"unknown"	castor oil	5 hours	fatal	[20]
19	$FeSO_4$ tabs.	4.8 gm.	none	4 hours	fatal	
18	$FeSO_4$ tabs.	13.2 gm.	lavage	5½ hours	fatal	
14	$FeSO_4$ tabs.	13.2 gm.	none	20 hours	fatal	
24	$FeCl_2$	5.3 gm.	transfusion	dismissed 2½ months	recovered, slight gastric changes	[21]
20	$FeSO_4$ tabs.	10 gm.	transfuse, lavage	16 weeks	fatal, stricture gastric	[22]
21	$FeSO_4$ tabs.	8.2 gm.	lavage $NaHCO_3$	4 hours	fatal	[23]

Further studies by Somers demonstrated that very large doses of iron, well above the normal intake, were required to kill experimental animals. He also showed that ferrous carbonate was approximately one-fourth as toxic as ferrous sulfate [25]. Animals treated with a variety of iron preparations seemed to be made toxic in direct proportion to the amount of iron that was quickly soluble. This was thought to be the explanation for the relatively low toxicity of ferrous carbonate, as compared with ferrous sulfate. Addition of manganese or copper had no further toxic effects. It can be estimated from these experiments that several hundred tablets of ferrous sulfate, containing 65 mg. each, would be required to kill an average man, if the minimum lethal dose for mice is used as a basis.

Critical reasoning based on an analysis of a relatively large number of cases of acute iron poisoning in children led Spencer to emphasize the fact that there is a lack of histologic evidence for liver failure as a cause of death [20]. He held that many symptoms were better explained on the basis of intracellular metabolic disturbances, and offered a formula of vitamins and amino acids that was designed to support oxidative mechanisms in the cells and contribute sulfhydryl groupings. Although some have tried this form of therapy there seemed to be no significant reduction in the mortality rate.

It has been suggested that the local effects of iron on the gastrointestinal tract might result in the release of unusual amounts of ferritin [17, 23]. This substance forms an essential part of the "mucosal block" proposed by Granick for regulating entry of iron into the body from the lumen of the gastrointestinal tract [26]. It was postulated that the corrosive changes in the duodenum resulted in destruction of the "mucosal block" and uncontrolled formation of ferritin, which could find its way into the circulation. This might cause the severe cardiovascular collapse seen in children since it is known that ferritin is a potent vasodepressor [27, 28]. Ferritin is also formed in liver, and additional amounts of ferritin could conceivably come from this site if large iron concentrations were allowed to reach the liver.

This concept was weakened when it was shown that destructive changes in the mucosal surfaces of the gastrointestinal tract were not necessary conditions for uncontrolled iron absorption. Dissociable iron salts rapidly cross the histologically intact intestinal mucosa of animals in amounts sufficient to cause severe intoxication and simulate the acute iron toxicity of children [29, 30]. Very high serum iron levels were reached within an hour after the iron salts were administered, and it was determined that the iron was, for the most part, in a ferric state and nondialyzable. A deep metabolic acidosis accompanied by low

pH values of the blood was observed, and because of an increase in lactic and citric acid it was proposed that there might be interference with the enzymes of the Krebs cycle. Blood carbon dioxide content was also lowered, and there was a characteristic increase in respiratory rate associated with metabolic acidosis. Cardiac output decreased as venous return was diminished, but blood pressure was maintained satisfactorily by arteriolar constriction until the final stages. The pulmonary changes that were noted seemed to be secondary to a capillary hemorrhagic tendency. A check for methemoglobin was negative. Sulfate ions may be an important factor in ferrous sulfate toxicity. This has been suggested [24], but further studies of this aspect have not been made.

Treatment and Prevention

It was apparent that treatment for acute iron poisoning in this group of cases left much to be desired. The following measures provide a logical plan of management.

1. *Rid the stomach of its contents.*—This may be done by inducing emesis and following with a thorough lavage, using a large-diameter tube that will allow undissolved tablets to be aspirated. The use of sodium bicarbonate for purposes of lavage may be helpful because it will reduce gastric acidity and may convert the iron to a less soluble state (ferrous carbonate).

2. *Institute immediate measures to overcome shock.*—Whole blood, plasma, or blood substitutes, such as dextran, are most useful during the first phase, and should be given in sufficient amounts to overcome cardiovascular collapse during the first few hours.

3. *Institute specific steps to neutralize or combine the iron remaining in the gastrointestinal tract and tissues.*—The study of the use of chelating agents in iron poisoning has not been published. In a recent experience with this form of therapy [32], there was a gratifying outcome. The use of BAL is controversial, and only a few patients have received it. Exchange blood transfusions have been suggested, and so has use of the artificial kidney. It is important, when considering the latter method, to remember that a large part of the iron in the serum remains in the ferric state and cannot be dialyzed [29, 30]. The use of cathartics and, particularly, enemas may be helpful since it is known that the iron may continue to be absorbed from the large bowel [29, 30].

4. *Other practical measures.*—A flat plate of the abdomen may give important indication of the number of tablets ingested, since iron tablets are radio opaque. It is conceivable that a large accumulation of tablets in an isolated segment of the bowel might be reached by surgical

intervention. Other supportive measures, including the use of oxygen, are important, but they are not substitutes for the above actions [31].

The best way to prevent acute iron poisoning in children is to warn parents of the hazard to their children, when the iron tablets are prescribed. It would be useful if tablets prescribed to pregnant women were allocated in small numbers rather than large prescriptions of a hundred tablets or more. Similarly, if iron tablets were packaged in tight wrappings the small child might be either delayed in opening the package or discouraged from eating a large number of tablets. Large prescriptions of iron tablets to every blood-bank donor should be discouraged unless proper warning accompanies the prescription. Finally, all medicines in the home should be carefully locked in a medicine cabinet inaccessible to children.

CONCLUSIONS

The fact that iron is an effective therapeutic agent in certain human disorders has led to its widespread prescription, usually as ferrous sulfate tablets. These tablets are attractive to small children between the ages of 2 and 4 years, and of the children poisoned by accidental ingestion of ferrous sulfate, 50 per cent have died.

Iron is rapidly absorbed in the gastrointestinal tract whether there is extensive injury to the bowel or not, and following absorption there is a very high level of serum iron with associated metabolic changes, probably secondary to intracellular effects. Death from iron poisoning is due to severe cardiovascular collapse. Treatment should be directed at overcoming shock and neutralizing or combining the iron remaining in the tissues and gastrointestinal tract.

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UTILIZATION OF INORGANIC IRON BY RUMINANTS AS INFLUENCED BY FORM OF IRON AND IRON STATUS OF THE ANIMAL[1]

C. B. Ammerman, J. M. Wing, B. G. Dunavant, W. K. Robertson, J. P. Feaster and L. R. Arrington[2]

University of Florida, Gainesville[3]

RUMINANTS respond to supplemental iron under certain feeding conditions. Calves may be deficient in iron at birth (Hibbs *et al.*, 1961), and iron-deficiency anemia has occured in calves when diets based on whole milk were fed (Thomas *et al.*, 1954; Blaxter *et al.*, 1957; Matrone *et al.*, 1957; Roy *et al.*, 1964). In each study cited, anemia was alleviated by iron supplementation. Forages grown on certain types of soil may not contain sufficient iron to promote normal hemoglobin formation. Becker and Henderson (1940) and Becker *et al.* (1965) reported that supplementation with iron corrected anemia occurring among grazing cattle in certain areas of Florida. In the same state milk yield was significantly reduced in dairy cows grazing newly developed pastures, but was maintained when cows were supplemented with iron (Wing and Ammerman, 1965).

Various forms of inorganic iron have been used as supplementary sources, but tests of comparative availability for ruminants have not been conducted. The present study was designed to compare the relative biological availability to ruminants of iron in ferric oxide (Fe_2O_3), ferric chloride ($FeCl_3$), ferrous carbonate ($FeCO_3$) and ferrous sulfate ($FeSO_4 \cdot XH_2O$), and to determine the influence of body iron stores on the absorption of orally administered radioactive iron.

Experimental

In a series of three experiments radioactive iron in the form of ferric oxide, ferric chloride, ferrous carbonate or ferrous sulfate was given as a single oral dose to calves and lambs. The radioactive ferric oxide used in Experiment I was prepared from Fe^{++} ferric chloride, and that used in Experiments II and III and the carbonate and sulfate sources for Experiment III were obtained from a commercial laboratory. All animals were given a 4-day adjustment period in metabolism stalls before they were dosed with labeled iron. The labeled iron compounds were administered by capsule just prior to a morning feeding. Ferric chloride was given in dilute HCl, and the three other forms of iron were given as the dry chemical. After the animals were dosed with labeled iron, total fecal and urinary excretions were collected, and blood samples were obtained by jugular puncture at varying intervals throughout the observation period. Hemoglobin was determined by the method of Cohen and Smith (1919) and tissue iron by the method of Sideris (1942).

All animals were slaughtered and the liver, heart, kidneys, spleen, and first and second ribs were measured for isotope content. Total iron also was determined in Experiment I. In the first two experiments samples were measured for radioactivity with a sodium iodide crystal well-type scintillation counter. Dry samples of feces and tissues were ashed at 600° C. and dissolved with dilute HCl. Radioactivity was determined in the urine as collected and in the blood serum following separation from the red blood cells. The red blood cells were prepared for counting by washing three times with an equal volume of physiological saline. In Experiment III all samples were measured for radioactivity with a 4 Pi liquid scintillation detector. The detector had a sample chamber 11.5 cm. in diameter by 30.4 cm. in length which permitted whole organs to be counted.

Data obtained were examined for significant differences by analysis of variance.

Experiment 1. Six dairy-type steer calves, referred to as "iron-depleted", were fed from birth whole milk supplemented with the following minerals expressed in milligrams per head daily: cobalt, 1; copper, 10; manganese, 25; zinc, 25; and iodine, 1. After 56 days the solid content of the diet was increased to 18%

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[2] Departments of Animal Science, Dairy Science, Radiology and Soils.

by addition of dry milk solids-not-fat. In addition 220 I.U. of vitamin A, 511 I.U. of vitamin D, 44 mg. of sodium chloride and 13 mg. of magnesium were fed daily per kilogram of bodyweight. Three similar calves not depleted of their iron stores were fed a standard mixed ration with hay or pasture. Seven days before the radioactive iron was administered and continuing until the end of the experiment, all calves received a ration consisting of 75% ground snapped corn and 25% pangolagrass hay and containing 72 ppm of iron on a dry matter basis. At an average age of 215 days and a bodyweight of 110 kg., three iron-depleted and the three nondepleted calves were each given a single oral dose of 400 μc. of Fe^{59} as ferric chloride. The three remaining depleted calves were dosed similarly with Fe^{59} ferric oxide. Approximately 73 mg. of stable iron were given with each dose. All animals were slaughtered 96 hr. after dosing.

Experiment II. Six dairy-type steer calves, which had received the diet low in iron described in Experiment I, were given radioactive iron at an average age of 172 days and a bodyweight of 91 kg. At this time average hemoglobin and hematocrit values were 9.6 gm. 100 ml. and 37.2%, respectively. Three steers each were dosed orally with 500 μc. of Fe^{59} as either ferric chloride or ferric oxide with 70 mg. of total iron per dose. The animals were slaughtered 168 hr. after dosing.

Experiment III. The relative absorption of Fe^{59} from ferrous sulfate, ferrous carbonate, ferric chloride and ferric oxide was determined with 24 wethers averaging 39.2 kg. in bodyweight. The sheep had received standard rations and had average hemoglobin and hematocrit values of 11.0 gm. 100 ml. and 36.2%, respectively. Twelve days prior to dosing, the animals were started on a ration containing 60% ground snapped corn, 20% cottonseed meal and 20% ground Bermudagrass hay. A uniform feed intake 900 gm. per head daily was established and maintained throughout the experiment. Six animals were used per treatment, and 150 μc. of Fe^{59} were administered orally per head. Total iron per dose depended on form and varied from 30 mg. for the chloride and oxide to 70 and 77 mg. for the sulfate and carbonate, respectively. The sheep were slaughtered 168 hr. after dosing.

Results

Experiment I. The effects of reduced dietary iron on blood hemoglobin and hematocrit and tissue iron concentration are shown in table 1. Hemoglobin and hematocrit values were lower ($P<.05$) in the iron-depleted calves. The depleted calves also had significantly less total iron in the spleen. Other tissues in depleted calves except the heart contained less iron, but differences were not significant.

TABLE 1. AVERAGE HEMOGLOBIN, HEMATOCRIT AND TISSUE IRON IN IRON-DEPLETED AND NONDEPLETED CALVES (EXPERIMENT I)

Item	Iron status	
	Depleted	Nondepleted
No. of animals	6	3
Hemoglobin, gm. 100 ml.	7.8*	10.8
Hematocrit, %	30.0*	37.0
Tissue (total Fe, ppm dry wt.)		
Rib	29	72
Muscle	75	85
Liver	169	233
Heart	329	223
Kidney	365	479
Spleen	664*	1067

* Significantly ($P<.05$) lower than the value for control calves.

An average of 63 and 54% of the oral Fe^{59} from ferric chloride was recovered in feces within 96 hr. for nondepleted and depleted calves, respectively. Only 14% of the Fe^{59} from ferric oxide was recovered from depleted calves. The calves receiving ferric oxide never excreted more than 2% of the oral dose during any 6-hr. period. Urine samples were counted for radioactivity, but no sample contained measurable activity.

Measurable levels of radioactivity were present in red blood cells of the depleted calves receiving ferric chloride from 36 to 48 hr. after oral administration (figure 1). Radioactivity in the red blood cells of the control calves receiving ferric chloride became measurable at a later time and was significantly lower at all periods of observation. Radioactivity in the red blood cells from depleted calves receiving iron oxide remained within the range of background variability. Activity was detected only in the serum of calves receiving ferric chloride, but levels of activity were so low that a clearance pattern after oral administration could not be established.

The tissue deposition of Fe^{59} is shown in table 2. The highest concentration of oral Fe^{59} was found in the liver, followed by the spleen, kidney, heart and rib. Iron-depleted calves, when compared with nondepleted calves, had

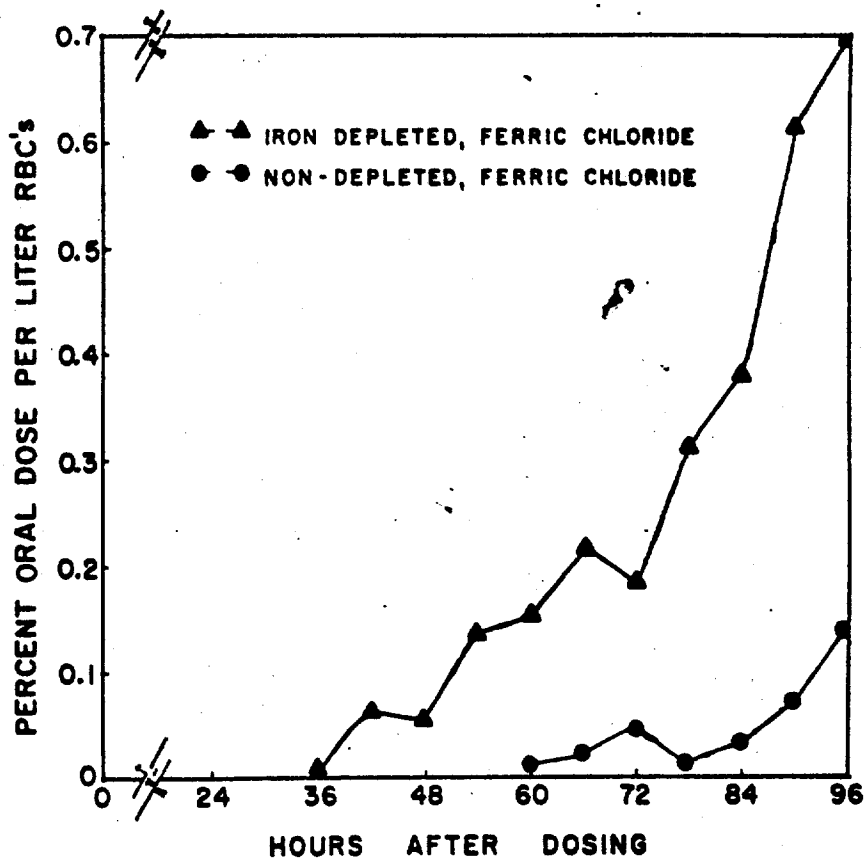

Figure 1. Red blood cell levels of iron[59] as influenced by iron status of the animal (Experiment I).

approximately five times as much Fe^{59} from ferric chloride in the liver. All tissues except rib indicated a significant effect of iron depletion on Fe^{59} deposition. No measurable radioactivity was present in any tissue from animals receiving ferric oxide.

TABLE 2. TISSUE DEPOSITION OF Fe^{59} FROM FERRIC CHLORIDE AND FERRIC OXIDE EXPRESSED AS PERCENT×10³ OF THE ORAL DOSE PER GRAM OF DRY TISSUE IN IRON-DEPLETED AND NONDEPLETED CALVES (EXPERIMENT I)[a]

	Iron status		
	Depleted		Nondepleted
Tissue	Chloride	Oxide	Chloride
Liver	169[c]	0[b]	33
Spleen	131[c]	0	21
Kidney	87[c]	0	27
Heart	57[c]	0	13
Rib	38	0	12

[a] Each figure represents an average of values from three animals.
[b] No measurable radioactivity.
[c] Significantly ($P < .05$) higher than value for nondepleted animals.

Experiment II. Calves receiving ferric chloride excreted an average of 82% of the oral dose by way of the feces in the 168-hr. experimental period, and those animals receiving ferric oxide excreted 64% of the oral dose. Fecal excretion values 96 hr. after dosing were 68 and 55% for ferric chloride and ferric oxide, respectively. The value of 68% for ferric chloride was somewhat greater than 54% obtained in Experiment I, and the excretion of ferric oxide (55% compared with 14%) was almost four times as great in Experiment II. The rumen contents contained 2.0 and 5.3% of the oral dose from calves receiving ferric chloride and ferric oxide, respectively. This suggests that the iron oxide powder may have passed from the rumen more slowly.

In contrast to the results obtained in Experiment I with a different ferric oxide preparation, measurable levels of Fe^{59} were ob-

served in the tissues of steers receiving ferric oxide (table 3). The Fe^{59} deposition, however, was significantly greater for each tissue when ferric chloride was administered than when ferric oxide was given. Radioactive iron was detected in the red blood cells in all calves approximately 48 hr. after dosing. Expressed as percent of oral dose per liter of packed red blood cells, averages of 0.71 for calves receiving ferric chloride and 0.19 for calves receiving ferric oxide were obtained at 168 hr. following dosing.

Experiment III. Fecal and urinary Fe^{59} excretion data are shown in figure 2. The total fecal recovery of Fe^{59} from the various sources, expressed as percent of the oral dose, varied from 86% for the ferric oxide to 98% for the ferric chloride with no significant difference between treatments. Less ($P<.05$) radioactive iron was excreted in the urine by those sheep receiving ferric oxide than by those receiving the other three iron sources. Approximately 0.09% of the oral dose was eliminated in the urine from the three sources except for ferric oxide.

The level of Fe^{59} in serum and red blood cells is shown in figure 3. The peak in serum activity occurred between 6 and 24 hr. after dosing. Statistical examination of the 24-through 96-hr. data revealed that the levels of radioactive iron from ferrous sulfate and ferric chloride were similar, that the activity from ferrous carbonate was lower ($P<.05$) than that of the sulfate, and that the response from ferric oxide was lower ($P<.01$) than that from the other three iron sources. Labeled iron accumulation in red blood cells was measurable 24 to 48 hr. after administration, and the relative levels of response to the various sources occurred in the same order as was found in the serum. The Fe^{59} uptake by the red blood cells was similar for the sulfate,

TABLE 3. TISSUE DEPOSITION OF Fe^{59} FROM FERRIC CHLORIDE AND FERRIC OXIDE EXPRESSED AS PERCENT$\times 10^3$ OF THE ORAL DOSE PER GRAM OF DRY TISSUE (EXPERIMENT II)*

Tissue	Forms of iron	
	Ferric chloride	Ferric oxide
Liver	211*	69
Spleen	114*	38
Kidney	102*	34
Heart	43**	9
Rib	113*	32

* Each figure represents an average of values for three animals.
* Significantly ($P<.05$) higher than value for ferric oxide.
** Significantly ($P<.01$) higher than value for ferric oxide.

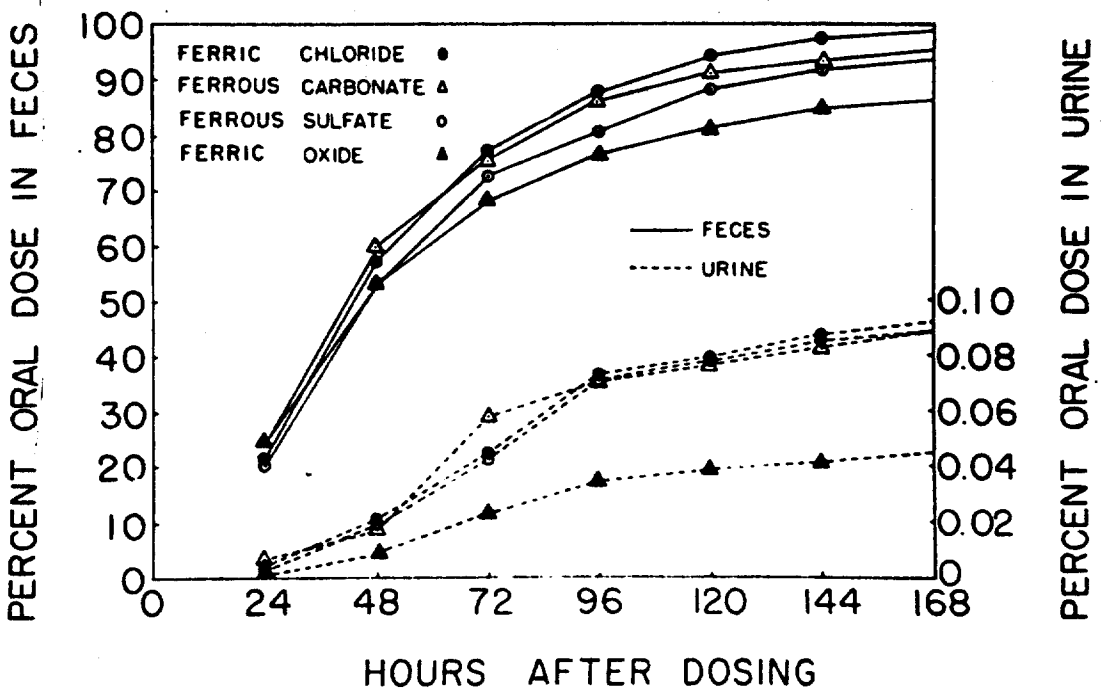

Figure 2. Accumulative fecal and urinary excretion of Fe^{59} as influenced by form of iron (Experiment III).

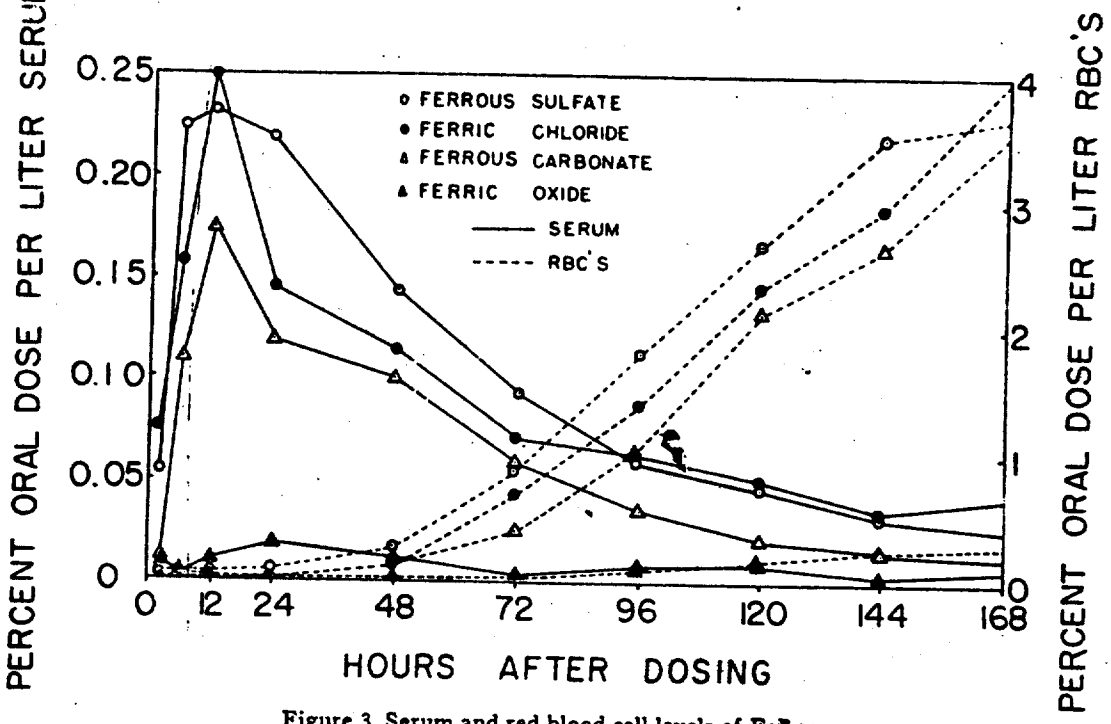

Figure 3. Serum and red blood cell levels of Fe^{59} as influenced by form of iron (Experiment III).

chloride and carbonate forms of iron, while the response for ferric oxide was lower ($P<.01$) than that for the other sources.

Tissue deposition of Fe^{59} (table 4) was similar for sheep receiving the sulfate, chloride or carbonate form of iron. There was less ($P<.01$) radioactivity present in the tissues from those animals which had received ferric oxide. Based on tissue deposition, the Fe^{59} in iron oxide was about 12% as available as that in the ferric chloride.

Discussion

Results obtained in Experiment I indicated greater absorption of iron when body stores were reduced. Similar responses have been reported in humans by Mitchell *et al.* (1960) and in humans and dogs by Moore *et al.* (1944). Djurdjevic and Georgi (1965) reported that the average absorption of orally-administered ferrous Fe^{59} was 6.5% in normal sheep and 20.2% in sheep made anemic by bleeding. These findings suggest the desirability of using iron-depleted animals in an iron availability assay.

The time lag of 24 to 48 hr. after oral dosing prior to detecting Fe^{59} in the red blood cells was dependent upon iron incorporation in the cells and is similar to the response obtained by Hansard *et al.* (1959) when calves were intravenously injected with Fe^{59}. In general the liver contained the highest concentration of Fe^{59}, followed by the spleen. This was especially true in the depleted calves, while in the nondepleted sheep, the concentration of Fe^{59} in the spleen approached or exceeded that found in the liver. In other work with calves it was found that liver contained the greatest concentration of intravenously

TABLE 4. TISSUE DEPOSITION OF Fe^{59} FROM DIFFERENT IRON COMPOUNDS EXPRESSED AS PERCENT×10³ OF THE ORAL DOSE PER GRAM OF FRESH TISSUE (EXPERIMENT III)[a]

Tissue	Forms of iron			
	Ferric oxide[b]	Ferrous carbonate	Ferric chloride	Ferrous sulfate
Liver	25	582	464	682
Spleen	53	660	443	601
Kidney	25	326	266	355
Heart	12	87	100	97
Muscle	4	20	17	24

[a] Each figure represents an average for 6 animals.
[b] All tissue values for ferric oxide were significantly ($P<.01$) lower than those for other forms of iron.

injected Fe^{59}, with the spleen and other organs containing Fe^{59} in considerably lower amounts (Hansard *et al.*, 1959).

A greater percent of the radioactive oral dose was accounted for by fecal excretion with the sheep in Experiment III than in the second experiment of the same duration with steers. Although the accumulative fecal excretion for individual animals was variable, the average excretion of 86 to 98% suggests only limited absorption of the orally-administered radioactive iron in the nondepleted sheep. The extremely high levels of iron excreted from the nondepleted sheep, plus the delayed rate of passage apparent with ferric oxide in Experiments I and II, suggest that differences between oral dose and fecal excretion may not be precise measures for evaluating iron absorption. In Experiment III the average percent of oral dose accounted for by incorporation of Fe^{59} into red blood cells was calculated. A blood volume of 6.2 ml. per 100 gm. of bodyweight (Hansard, 1956) and the average hematocrit value obtained with the serial blood samples were used in making the calculations. The values obtained were 0.2, 2.7, 2.9 and 3.2% for the oxide, carbonate, sulfate and chloride, respectively.

Iron from ferric oxide was significantly less available than that from other sources in all three experiments. Based on tissue deposition data, ferric oxide was approximately 0 and 30% as effective as ferric chloride in the two experiments with steers and 12% as effective as ferric chloride in the experiment with sheep. The method of counting Fe^{59} in the tissues in Experiment III may have allowed detection of less Fe^{59} from ferric oxide than was possible in the earlier experiments. The samples of Fe^{59} ferric oxide represented separate preparations for each experiment, and it is suggested that an incomplete conversion of Fe^{59} into the oxide form may explain the apparent higher availability obtained for this form of iron in Experiment II. In a study by Pickett *et al.* (1961) iron from ferric oxide was shown to be relatively unavailable to young pigs. In the same study ferrous sulfate gave the highest gains and hemoglobin values, while the response to iron carbonate was intermediate between that obtained for ferrous sulfate and that obtained for ferric oxide.

Iron is considered to be absorbed only in the ferrous state, although there appear to be species differences in the ability to reduce ferric to ferrous iron (Underwood, 1962). Moore *et al.* (1944) reported that human subjects absorbed 1.5 to 15 times as much ferrous iron as ferric iron, while dogs either absorbed both forms to a comparable degree or showed preferential absorption of ferrous salts. The two forms of iron have been reported to be equally effective for rats (Underwood, 1938). In Experiment III with sheep, Fe^{59} from ferrous sulfate and ferric chloride appeared in the serum more quickly and remained higher throughout the observation period than did the Fe^{59} from ferrous carbonate. This response was reflected in red blood cell radioactivity. Ferric oxide yielded little Fe^{59} in either serum or red blood cells. Tissue concentration of Fe^{59} from ferrous sulfate was higher than that from ferrous carbonate and ferric chloride, but the differences were not significant. Significantly less Fe^{59} was deposited from ferric oxide.

Relative solubility of the iron compounds, as well as degree of reduction of ferric to ferrous iron, may have influenced utilization of the various forms of iron. Both ferrous sulfate and ferric chloride are very water soluble, while ferrous carbonate is only slightly soluble and ferric oxide relatively insoluble.

Summary

Calves and sheep were used to determine the relative biological availability of radioactive iron administered orally in the form of ferric oxide, ferric chloride, ferrous carbonate and ferrous sulfate. In addition, calves were used to determine the influence of body iron stores on the absorption of orally administered radioactive iron. Ferrous sulfate, ferrous carbonate and ferric chloride ranked in decreasing order of availability, but were not significantly different, when evaluated on the basis of tissue Fe^{59} deposition. Ferrous sulfate yielded serum Fe^{59} levels which were significantly higher than those for carbonate, but not different from those for ferric chloride. Iron in ferric oxide was significantly less available to both calves and sheep than iron in the other compounds. In one of the three experiments, measurable levels of radioactive iron were not detected in tissues of calves receiving Fe^{59} ferric oxide. Tissues from iron-depleted calves receiving Fe^{59} ferric chloride contained three to five times as much radioactivity as corresponding tissues from nondepleted calves receiving the isotope in the same compound.

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ON THE GROWTH-PROMOTING INFLUENCE OF VARIOUS INORGANIC IRON COMPOUNDS, AND THE INCREASED IRON CONTENT OF THE BODY FOLLOWING THE ADMINISTRATION OF THE ACTIVE IRON OXIDE, "SIDERAC"

by

A. Bickel

(From the experimental-biological division of the Pathological Institute of the University of Berlin)

(Entered on May 25, 1928)

The fact that a growth-promoting influence can be ascribed only to inorganic iron compounds, and not to the organic ones, has been established through the works of Abderhalden (1). Therefore, the growth experiments described in the following were conducted using solely inorganic iron compounds. The following iron compounds were those tested:

1. "Siderac", the ferric oxide of Baudisch, strongly magnetic, and benzidine active in an acid medium.

2. A likewise strongly magnetic, benzidine active ferric oxide which, in a manner similar to that of the oxide of Baudisch, was produced by oxidation of magnetite. However, a different magnetite was used for this oxide.

The magnetites are ferric oxiduloxides, which can also partially be obtained from hydrates. Lerfort's magnetite, from which Siderac is produced, contains ferrous and ferric iron in equal proportion and a great deal of water as well, partly adsorbed, and partly chemically bound. Because of the diamagnetism of the water and also the loose quality of the magnetite, this abundant water content brings about the condition that, in its water complex, this magnetite cannot be raised above the level of fluidity by a strong magnet. In the case of magnetites poorer in water content, this succeeds immediately. Such a magnetite, poorer in water and with a ferrous content greater than that of the Lerfort magnetite, served in the production of the second oxide.

The end product was just as magnetic as the siderac, and also revealed a benzidine reaction. In spite of this, an identity of the two oxides cannot, of course, be claimed.

3. A strongly benzidine active but unmagnetic ferrous ferric carbonate. The experiments with this compound were carried out in part by Dr. Remesoff (Leningrad) in this laboratory. Prof. Völtz also kindly allowed some experiments to be conducted at the Animal Breeding Institute of the University of Königsberg. Remesoff has already reported the results of his experiments

with this carbonate, as well as those of several siderac experiments, in Russian at the Pathologists' Congress in Kiev in 1927.

The arrangement of the experiments was as follows. For a part, we used rats of approximately the same age and the same sex, but from different litters; for the other part, we used rats of the same sex from the same litter. In most of the experiments, all the animals were fed with dried rolls and fresh baby milk.. In a morning portion, they received a little of this nourishment as well as the respective dosage of iron; the control animals were given the same amount of nourishment only. After this portion had been completely consumed, and the iron animals had received their daily ration of iron, such an amount of food was placed in the cages of all the animals, so that on the following morning food scraps still remained, and were then removed. On a certain weekday each animal was weighed. The total weight of each group was then ascertained, in order ultimately to establish the percentage of weight gain based on the initial weight. In a rather extensive series of experiments, the iron content of the body was established in some of the animals. These were double experiments with rats of the same sex from the same litter. Animals of the same sex were separated from each litter and fed in the manner described above, half of them receiving the dose of siderac, half not. After the observation of growth was concluded, the rats were killed with chloroform vapor, washed with ether and, after the stomach and intestine had been removed, placed in large numbered porcelan pots. Then the pots were weighed and kept at a temperature of 105 to 110° C until the weight remained constant at around 1/10 g. Then the contents of the pots were incinerated, the ashes dissolved in forensic hydrochloric acid, and the iron was determined in the solution through electrometric titration with sodium bromate.

Records of Experiments

Experiment 1

Masculine white rats of different litters aged about 6 weeks. The animals were kept together and fed in groups of 10 (9).

1.	2.	3.	4.	5.	6.	7.
1	[illegible]	[illegible]	626 g 22. XI. 1926	1597 g 24. I. 1927	9	155,1
2	9	10 mg Fe++	517 g 22. XI. 1926	1360 g 24. I. 1927	9	163,0
3	10	--	624 g 22. XI. 1926	1452 g 24. I. 1927	9	132,6

Key:
1.= group 2.= number of animals 3.= daily dose per animal
4.= initial weight and date of commencement of experiment
5.= end weight and date of conclusion of experiment
6.= duration in weeks
7.= weight gain %

Results of the experiment. Compared with the controls, siderac effected an acceleration of growth of 23% within 9 weeks, the ferrous ferric carbonate one of 31%. With reference to the iron content, the doses of the different iron compounds were nearly equal. Siderac contains 70% iron, the ferrous ferric carbonate 36%. (This ferrous ferric carbonate, which exercised an especially favorable influence here, had caused no change in the urine quotient C/N in the metabolism experiments of Rosenfeld (2).

The marked weight gain of the iron carbonate animals induced us to seek verification from another source. This was carried out in the Animal Breeding Institute of the University of Königsberg under the direction of Prof. Völtz. The iron carbonate which was sent to Königsberg for this purpose had already been preserved for quite some time in the laboratory and had partly decomposed; it had turned red, but still gave evidence of undiminished intensity of benzidine reaction. Under the impression that the intensity of the benzidine reaction is connected with the effect on growth, an impression which could well be garnered from the results of the first experiment, we did not prepare a fresh preparation of a light color. The use of this decomposed preparation appears to have been the reason for the negative outcome of the Königsberg experiments.

Experiment 2

Course of the rat experiment in Königsberg with 10 mg Fe-preparation (ferrous ferric carbonate, decomposed, but strongly benzidine active) as a daily supplement to the basic diet.

For the comparison, rats of the same age and same weight were selected; of these, nos. 1, 2, 3, 4, 9, and 10 came from the same litter. At the time of the commencement of the experiment, all the animals were about 5 weeks old. Immediately after weaning, they underwent a two-week preliminary period (without Fe-supplement) with the basic diet; during this period, rats nos. 7 and 8 perished.

The main experiment began in the eighth week. Each animal was kept separate. In their daily ration of the basic diet consisting of 12.5 ccm milk, 3.5 g rolls, 8 g grist (2 parts wheat, 1 part oats, 1 part rye) , nos. 1 and 6 received 10 mg iron preparation each. Nos. 9 and 12 received only the basic diet, in order to serve as control animals. The animals were weighed once a week, in the morning on an empty stomach.

All the experimental animals consumed the Fe-preparation given them with the milk and rolls, quantitatively. In the first two weeks, only rat no. 4 left some milk with Fe standing.

The grist remains were collected separately from each animal and at the end were subtracted from the amount of food given every day. The initial and end weights, the weight gains (absolute and percentual) and the food consumed by the individual animals as well as the group average, are explained in the

following table.

	1.	2.	3.		4.		
			a) Zunahme	b)	a) Verzehrtes Futter		c)
	Anfangsgewicht	Endgewicht	absolut	in % des Anfangsgewichts	Milch	Semmel	Schrot
	g	g	g		ccm	g	g
			Gruppe 1.				
A.	Versuchsdauer 50 Tage. Zusatz von 10 mg Fe je Tag. (Zwei männliche, vier weibliche Tiere, darunter vier Wurfgeschwister.)						
B.	Durchschnitt 58,3	114,6	56,3	96,6	625	175	335,3
			Gruppe 2.				
C.	Versuchsdauer 50 Tage, ohne Fe-Zusatz. (Zwei männliche und zwei weibliche Tiere, darunter zwei Wurfgeschwister.)						
B.	Durchschnitt 59,0	121,3	62,2	105,4	625	175	356,5

Key:
1.= initial weight 2.= end weight
3.= gain a) absolute b) in % of initial weight
4.= food consumed a) milk b) rolls c) grist
A.= Duration of experiment 50 days, dose of 10 mg Fe each day (two male, four female animals; of these, four from the same litter)
B.= average
C.= Duration of experiment 50 days, no Fe added. (two male and two female animals; of these, two from the same litter)

Result: The Fe-preparation tested in this experiment with rats revealed no growth-promoting properties in a daily dosage of 10 mg as a supplement to the basic diet.
The food consumption and weight gains of the Fe-rats were even, in comparison to the control rats, somewhat reduced.

Course of the rat experiment in Königsberg with 20 mg Fe-preparation (ferrous ferric carbonate, decomposed, but strongly benzidine active) as a daily supplement to the basic diet.

The rats used for the experiment were of the same litter and about 5 weeks old at the time of the commencement of the experiment. After weaning they underwent a preliminary period of 10 days on the basic diet without Fe-supplement. Three days after the conclusion of the preliminary feeding, rat no. 4 perished.

Rats nos. 1 through 5 received 20 mg of the iron preparation each daily. The basic diet consisted on the average of 12.5 ccm milk, 3.5 g rolls, 7.2 g grist. For exact dosing, the iron preparation was mixed with technical casein. The control animals received a corresponding casein supplement.

The Fe was given together with the milk and rolls. It was consistently quantitatively consumed.

The grist remains of each animal were collected and subtracted at the end of the experiment from the amounts of food given. The initial and end weights, the weight gains (absolute and percentual) and the food consumed by the individual animals as well as the group average, are explained in the following table.

Group 3.
Duration of experiment 34 days, dose of 20 mg Fe-preparation per head and day.

	1.	2.	3.		4.		
			a) Zunahme	b)	a) Verzehrtes Futter		c)
	Anfangs-gewicht g	End-gewicht g	absolut g	%	Milch ccm	Semmel g	Schrot g.
Durchschnitt	56.8	108.0	51.25	90.2	420	120	211
-	55.6	108,1	53	95.3	420	120	218

Key:
1.= initial weight 2.= end weight
3.= gain a) absolute b) percentual
4.= food consumed a) milk b) rolls c) grist

Note: All the animals belong to the same litter. Preliminary period 10 days without Fe-supplement. Rat no. 4 died 3 days after the conclusion of the preliminary period.

Experiment 3

Male white rats of different litters mixed together; age about 10 weeks.

1.	2.	3.	4.	5.	6.	7.
Gruppe	Zahl der Tiere	Tagesgabe pro Tier	Anfangsgewicht und Versuchsbeginn	Endgewicht und Schluß des Versuches	Dauer Wochen	Gewichtszunahme %
1	5	—	605 g 27. II. 1927	1983 g 1. V. 1927	9	75.7
2	5	2 mg Ferrioxyd 2	605 g 27. II. 1927	1945 g 1. V. 1927	9	72.7

Key:
1.= group 2.= number of animals 3.= daily dose per animal
4.= initial weight and date of commencement of experiment.
5.= end weight and date of close of experiment.
6.= duration in weeks
7.= weight gain %

Result of experiment. The addition of oxide 2 to the basic diet caused absolutely no acceleration of growth.

Experiment 4

Male white rats of different litters mixed together; age of about 7 weeks.

1.	2.	3.	4.	5.	6.
Zahl der Tiere	Zulage pro Tier täglich	Anfangsgewicht und Versuchsbeginn	Endgewicht und Schluß des Versuchs	Dauer Wochen	Gewichtszunahme %
10	5 mg Ferrioxyd 2	702 g 13. XII. 1926	1531 g 14. II. 1927	9	118

Key:
1.= number of animals 2.= daily dose per animal
3.= initial weight and date of commencement of experiment
4.= end weight and date of close of experiment
5.= duration in weeks
6.= weight gain%

Results of experiment. Group 3 of experiment 1 lends itself to comparison here; in that group, a weight gain of 132.6% appeared within the same amount of time in rats aged about 6 weeks at the beginning of the experiment. The weight gain of 118% in rats of about 7 weeks, established in experiment 4, is entirely comparable to the weight gain of animals of corresponding age, kept on the same diet without iron supplement.

Experiment 5

Male white rats, that is the animals of experiment 4, which was cut short on February 14, 1927, were divided into two groups of five each; on February 28, 1927 a new experiment was begun on them.

1.	2.	3.	4.	5.	6.	7.
Gruppe	Zahl der Tiere	Zulage pro Tier täglich	Anfangsgewicht und Versuchsbeginn	Endgewicht und Schluß des Versuchs	Dauer Wochen	Gewichtszunahme %
1	5	—	855 g 28. II. 1927	1188 g 16. V. 1927	11	39.0
2	5	5 mg Ferrioxyd 2	805 g 28. II. 1927	1140 g 16. V. 1927	11	36,6

Key: see experiment 3.

Results of experiment. The growth of the animals is influenced neither by the addition nor the omission of ferric oxide 2 in the food.

From experiments 3, 4, and 5, which concern a benzidine active, strongly magnetic ferric oxide, the production of which is rather different from that of the oxide of Baudisch, it can be concluded that this oxide, as a supplement to the diet in a quantity of 5 mg per animal and day, does not influence the body growth of young rats. (This iron oxide was also tested by Rosenfeld (2) in metabolism experiments and revealed no influence on the urine quotient C/N, while the iron oxide siderac, according to Baudisch, alters the urine quotient in such experiments.)

Experiment 6

Male white rats of different litters mixed together; age about 7 weeks.

1. 2. 3. 4. 5. 6. 7.

Gruppe	Zahl der Tiere	Zulage pro Tier täglich	Anfangsgewicht und Versuchsbeginn	Gewicht am	Dauer Wochen	Gewichtszunahme %
1	6	5 mg Siderac	465 g 8. X. 1926	10. XII. 1926 1170 g	9	151,6
				21. I. 1927 1270 g	15	173,1
2	3	5 mg inaktiviert. Siderac	256 g 8. X. 1926	10. XII. 1926 630 g	9	146,1
				21. I. 1927 705 g	15	175,4
3	3	Ohne Eisenzulage	244 g 8. X. 1926	10. XII. 1926 585 g	9	139,8
				21. I. 1927 661 g	15	170,4

Key: see experiment 3.

Results of experiment. In young rats, a supplement of the active magnetic ferric oxide of Baudisch (siderac), effected a clear weight gain of 11.8% in the first 9 weeks, or 5.5% as compared to the rats which received no iron supplement, or those which received a supplement of siderac which had lost its magnetism and activity due to extended heating at 400° or more. In the following 6 weeks, however, the difference in growth was completely balanced out. The growth-promoting influence was, in this experiment, only a temporary one.

Experiment 7

The effect of siderac was now tested on a larger number of white rats. These experiments lasted from June 28, 1927, until October 18, 1927, thus 112 days or 16 weeks. The iron animals initially received 5 mg siderac daily; begining on July 23, the doses of siderac were increased ten times. The 33 rats of these experiments came from six litters; animals of the same sex and of the same litter were consistently used for the main and control experiments. The results are compiled in the following table.

1. 2. 3. 4. 5. 6. 7.

Gruppe	Zahl der Tiere			Zulage	Gesamtanfangsgewicht g	Gesamtschlussgewicht g	Zunahme in 16 Wochen %
1	1	2	Wurfgeschwister	Siderac	76	190	111
1a	2	3		—	167	325	97
2	2	3	Wurfgeschwister	Siderac	79	315	298
2a	1	2		—	40	140	250
3	1	2	Wurfgeschwister	Siderac	44	195	343
3a	2	3		—	88	370	320
4	2	3	Wurfgeschwister	Siderac	122	435	257
4a	1	2		—	70	220	214
5	1	2	Wurfgeschwister	Siderac	59	160	171
5a	2	3		—	120	300	150
6	2	3	Wurfgeschwister	Siderac	87	290	234
6a	1	2		—	42	165	293
7	1	2	Wurfgeschwister	Siderac	46	215	367
7a	2	3		—	95	430	352
8	2	3	Wurfgeschwister	Siderac	105	430	310
8a	1	2		—	52	205	295
9	1	2	Wurfgeschwister	Siderac	47	150	220
9a	2	3		—	103	315	206
10	3	5	Wurfgeschwister	Siderac	123	665	440
10a	3	5		—	139	595	327

Key to table on page 7:
1.= group 2.= number of animals 3.= supplement
4.= total initial weight 5.= total end weight
6.= gain in 16 weeks 7.= from the same litter

From these figures it is evidenced that, with the single exception of group 6, the weight gains of the iron animals are greater than those of the control animals. The influence of the siderac supplement becomes clearer when, forgoing the separation into animals of the same litter, one divides the material of the experiment into two main and two control groups, as shown in the next table. Here we also have in the last column the average relative iron content, which was established for a large number of the animals by analysis.

		1. Anzahl	2. Gesamtanfangsgewicht g	3. Gesamtschlußgewicht g	4. Gewichtszunahme in 16 Wochen %	5. Durchschnittl. Eisengehalt Fe ‰
A.	Weibl. Kontrolle	8	472	1245	164	0,0881
a.	„ Sideracgruppe . .	7	348	1057	203	0,122
B.	Männl. Kontrolle	9	444	1820	310	0,0671
b.	„ Sideracgruppe . .	9	440	1940	340	0,0907

Key:
1.= number (of animals) 2.= total initial weight
3.= total end weight 4.= weight gain in 16 weeks
5.= average iron content
A.= female controls a.= female siderac group
B.= male controls b.= male siderac group

In contrast to the results of experiment 6, in experiment 7 a pronounced effect of the siderac supplement can be recognized not only at the beginning of the experiment, but also during the entire duration of the experiment, although this experiment lasted considerably longer. The effect is especially clear in the case of the female animals and amounts to 39% in favor of the iron animals, while in the case of the male animals, a 30% increase for the iron animals presents a lesser, but nonetheless unmistakable effect.

It results further from experiment 7, that a part of the iron fed to the animals is re-absorbed, that is, in such quantities, that the siderac animals receive about 40% more iron than the controls. During the determination of the absolute iron values, this increase in iron revealed itself in the fact that there were consistently found at least 2 to 3 mg more iron in the iron animals than in the controls. The analysis revealed the iron values exactly to .1 mg, only the 1/100 mg were undetermined. Thus the increased iron content of the siderac animals lies far beyond the margin of error. The somewhat greater relative iron content of the female animals might be explained by the fact that they were without exception smaller, and therefore -- presupposing a re-absorption as great as in the case of the male animals -- attained higher percentages with approximately the same absolute quantities.

RESULTS

If one surveys the results of the cited experiments, one is led to the conclusion, that a promotion of the growth of rats nourished with a diet sufficient in every respect, can be effected through feeding with certain iron compounds. However in addition, as results from the imperfect reproductibility of such experiments, a coinciding of circumstances which are as yet unknown is necessary. This can be caused by the condition of the experimental animals, which, depending upon their age, origin and perhaps even depending on the season, can suit the purposes of the experiment differently. Another point of uncertainty can be found in the quality of the administered iron. Especially instructive in this respect is the comparison between the oxide of Baudisch and oxide 2 which, produced from another magnetite, can hardly be distinguished immediately from the first. While siderac (the ferric oxide of Baudisch) at least reveals a considerable capability of accelerating growth, the likewise magnetic and likewise benzidine active iron oxide 2 reveals nothing of the sort. According to this, the production of iron compounds must depend upon details whose significance is still completely obscure. The variable results of the experiments with ferrous ferric carbonate can be interpreted in the same sense, although in this case, at least, the various results must with all probability be attributed to clearly recognizable differences in the nature of the preparations. It is further possible to enrich the body iron content considerably through administration of siderac.

LITERATURE

Literatur.

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ACUTE FERROUS SULFATE POISONING

Report of a Nonfatal Case

Robert E. Birk, M.D.,* Detroit, Mich., and Sam K. Stallard, M.D.,**
Reidsville, N. C.

A CASE of acute ferrous sulfate poisoning is presented. A review of the literature reveals that most patients who received an equivalent amount did not survive. Many patients who ingested much less than this patient died. A brief review of the literature is included.

CASE REPORT

S. B. (Henry Ford Hospital No. 720432), a 26-month-old white female infant, was admitted to The Henry Ford Hospital on June 25, 1953, at 2:30 P.M. Approximately two and one-half hours prior to admission the patient had ingested sixty-five proprietary iron capsules containing a total of 13 Gm. of ferrous sulfate. Of the original one hundred capsules in the bottle, twenty-five were found on the floor. Ten had previously been used by the mother. The composition of this preparation was ferrous sulfate, 0.2 Gm., and molybdenum oxide, 3.25 mg.

The patient had been left alone, temporarily, and when found, shortly after ingestion, was very lethargic and retching violently. Her legs were soiled with a large amount of black feces.

The past history was negative except for tonsillitis at 6 months of age.

Physical examination revealed a well-developed, well-nourished, lethargic white female infant, who responded minimally to external stimuli. The temperature was 99.4° F. rectally, the pulse was 150, and respirations were 26 per minute. The blood pressure was 90 systolic and 60 diastolic.

The child appeared acutely ill with shallow respirations, thready pulse, marked pallor, and weakness. The head, eyes, ears, nose, and throat were normal. The chest was clear to percussion and auscultation. The heart rate was rapid with sinus arrhythmia but otherwise normal. Abdominal examination was not remarkable. Neurological examination revealed hypoactive deep tendon reflexes plus lethargy.

Immediately upon admission the child was placed in an oxygen tent. A Levin tube was passed, and normal saline was started intravenously. The stomach was lavaged with 500 c.c. of normal saline, bringing back greenish material with occasional flecks of bright red blood. No fragments of the capsules were recovered.

Following the lavage, 1 ounce of mineral oil and 8 ounces of 50 per cent milk-50 per cent cream mixture were left in the stomach. Ten ounces of a saturated solution of sodium bicarbonate were then started through the tube by slow drip.

The following treatment, modified after Spencer's,[1] was then instituted. A saline intravenous drip containing vitamin B complex was continued for approximately forty-eight hours. One hundred cubic centimeters of plasma was given intravenously the afternoon of admission.

The Levin tube was left in place and through this 4 ounces of milk-cream mixture and 0.2 Gm. of bismuth carbonate were given every four hours. Methionine, 500 mg., and Eprolin, 5 mg., were given every twelve hours through the tube.

*Resident in Medicine, The Henry Ford Hospital.

**Formerly Resident in Medicine, The Henry Ford Hospital.

In addition, 30 mg. of dimercaprol (BAL) was given intramuscularly every four hours for forty-eight hours and every twelve hours on the third day.

This regimen was continued until the afternoon of the third hospital day when the Levin tube was removed and the medications were discontinued. Prophylactically, aqueous procaine penicillin, 300,000 units, and streptomycin, 100 mg., were given every six hours for the hospital stay.

The child improved rapidly, and within six hours she was fairly active but was still having many large tarry stools. There was no retching or vomiting after admission. Blood pressure and vital signs became and remained stable, and eighteen hours after admission she was removed from oxygen. She had no further difficulty except for a short period of lethargy and a mild drop in blood pressure approximately thirty-six hours after admission. The tarry diarrhea cleared forty-eight hours after admission, and she began to pass normal stools.

On the third hospital day she was given a soft diet and supplemental vitamins. The urine was tested three times a day from the third to sixth day for sugar and acetone because of the report on the second day, and only once did it give a one-plus Benedict's reaction. She tolerated the soft diet well, became completely asymptomatic, and was discharged on the sixth day.

Laboratory results were as follows:

First Day (four hours after ingestion): Hemoglobin was 13.7 Gm.; red blood cells 4,800,000; white blood cells 30,800 with 86 per cent neutrophils, 13 per cent lymphocytes, and 1 per cent monocytes. Carbon dioxide combining power was 22.6 meq. per liter; chloride 108 meq. per liter; serum iron 4.0 mg. per cent (normal in this laboratory, 0.1 mg. per cent).

Second Day: Hemoglobin was 12.4 Gm.; red blood cells 4,900,000; white blood cells 10,600 with 61 per cent neutrophils, 4 per cent eosinophils, and 35 per cent lymphocytes. Urine: specific gravity 1.027, alkaline, negative albumin, one-plus Benedict, positive acetone and diacetic acid; spun sediment: 4 to 6 leukocytes per field and numerous epithelial cells. Serum iron was 2.0 mg. per cent.

Third Day: Serum iron was 0.92 mg. per cent.

Fourth Day: Hemoglobin was 13.9 Gm.; red blood cells 4,800,000; white blood cells 7,200 with 44 per cent neutrophils, 2 per cent eosinophils, and 54 per cent lymphocytes. Stool: normal color and consistency, three-plus phthalein test for occult blood. Serum iron was 0.38 mg. per cent.

Fifth Day: Serum iron was 0.40 mg. per cent. Fasting blood sugar was 92 mg. per cent.

Sixth Day: Hemoglobin was 13.3 Gm.; red blood cells 4,500,000; white blood cells 8,150 with 27 per cent neutrophils, 1 per cent eosinophils, and 72 per cent lymphocytes. Serum iron was 0.87 mg. per cent.

A telephone conversation was had with the mother one month from the day of admission. She reported the child to be in excellent health.

DISCUSSION AND REVIEW OF THE LITERATURE

Although many cases of oral iron poisoning were reported in the last half of the nineteenth century,[8, 11] the earliest case reported in the modern literature was that by Thomson.[23] This occurred in May, 1944, and was reported in 1947. Since then thirty cases, summarized in Table I, have been noted, with a fatality rate of 53.3 per cent.

Most modern textbooks of pediatrics still refer only to mild gastrointestinal symptoms as toxic effects of oral iron.[6] Forbes[10] and Spencer[21] quote earlier authors in reporting encephalopathy apparently due to oral iron therapy.

Most of the cases reported in the British literature involved ingestion

of a proprietary compound composed of 0.2 Gm. of ferrous sulfate, 2.5 mg. of copper sulfate, and 2.5 mg. of manganese sulfate. Forbes,[10] by animal feeding experiments, found the ferrous salt to be the toxic agent. Ferrous sulfate undoubtedly was the noxious substance in our case. Not much in-

TABLE I

AUTHOR	CASE NO.	AGE (MO.)	SEX	DOSE (GM.)	TREATMENT	RESULT
Branch[9]	1	29	M	18.0-22.5	Lavage, O_2, heat, suction, IV fluid, blood	Died, 4½ hr.
Duffy and Diehl[2]	2	15	F	4.9-6.4	IV saline	Recovered
	3	18	M	4.9	Lavage, IV fluid, plasma, blood, penicillin	Recovered
	4	26	F	9.75-13.0	Lavage, heat, O_2, IV fluid, caffeine	Died, 4½ hr.
Forbes[10]	5	39	M	10.0*	None	Died, 53 hr.
	6	12	M	6.0-7.0*	Heat, milk, Nepenthe, O_2, atropine, penicillin	Died, 30 hr.
Foucar[11]	7	26 yr.	M	113.5	Lavage, O_2, blood, artificial respiration	Died, 3 hr.
Lancet[8]	8	16	F	8.0*	?	Died
Lindquist[12]	9	24	F	5.34†	Blood, penicillin	Recovered
	10	?	?	10.68†	?	Died
Murphy[13]	11	30	F	15.0	Lavage, Na bicarbonate, AlOH gel, penicillin, milk	Recovered
Prain[14]	12	11	F	?*	Lavage, Na bicarbonate, sulfa, bismuth carbonate	Died, 39 hr.
Roxburgh[15]	13	16	M	6.0-9.75	Lavage, $MgSO_4$, IV fluid, penicillin, BAL	Recovered
Shoss[16]	14	14	F	16.3-24.4	Lavage, IV fluid, milk, O_2, penicillin, BAL	Recovered
Smith, J.[17]	15	21	M	8.2*	Lavage, Na bicarbonate	Died, 4 hr.
Smith, R.[18]	16	17	F	6.5?	Coramine, O_2, plasma, methylene blue, IV fluid	Died, 11 hr.
Spencer[21]	17	21	F	10.8*	Lavage, Na bicarbonate, bismuth carbonate (serum iron: 4 hr., 3.3 mg.; 3 days, 0.26 mg.)	Recovered
	18	23	M	5.8*	Saline, bismuth carbonate, vitamins,‡ IV fluid (serum iron: 4½ hr., 3.42 mg.; 6 days, 0.33 mg.)	Recovered
	19	11	M	1.4-1.8*	Lavage, bismuth carbonate, vitamins‡	Recovered
	20	20	M	0.6*	None	Recovered
	21	12	M	?*	Saline, castor oil	Died, 4 hr.
	22	19	F	3.0-3.2*	Saline	Died, 4 hr.
	23	18	M	8.8*	Lavage, stimulants	Died, 5½ hr.
	24	14	F	8.0*	Castor oil, kaolin	Died, 26 hr.
Swift[22]	25	19	F	?	IV fluid, blood, BAL, Penicillin, streptomycin, O_2, vitamin K, Amphojel	Died, 40 hr.
Thomson[23]	26	16	F	5.2*	Lavage, Na bicarbonate, Nepenthe, bismuth carbonate	Died, 21 hr.
	27	24	M	1.6*	Magnesium hydroxide, bismuth mist., IV fluid, milk	Recovered
Thomson[24]	28	54	F	0.8*	Bismuth carbonate	Recovered
	29	19	M	2.0*	Syrup of figs, lavage, Na bicarbonate, BAL	Recovered
	30	30	M	2.0-4.0*	Syrup of figs, lavage, magnesium sulfate	Recovered

*Also 12.5 mg. copper sulfate and 12.5 mg. manganese sulfate per gram of ferrous sulfate.
†Ferric chloride.
‡See text.

formation is available as to the toxicity of molybdenum, but Fairhall[9] states: "No toxic effects in workers exposed to the dust and fumes of molybdenum and its common compounds have been reported."

Notable in this case was recovery after such a large dose. Only two patients with a larger amount survived. Murphy and associates[13] state that their patient never appeared acutely ill. Shoss[16] treated his patient in a manner similiar to ours.

Treatment was modified after Spencer,[21] as recommended in the JOURNAL.[7] He advocated immediate production of vomiting, followed by gastric lavage, leaving sodium bicarbonate in the stomach. He also suggested bismuth carbonate, 0.2 Gm., every four hours, and the following vitamin mixture: 10 mg. of aneurin hydrochloride, 30 mg. of nicotinamide, 10 mg. of riboflavin, 15 mg. of tocopherol, and 500 mg. methionine. (This to be multiplied by the age in years and divided into three doses per day.) Somers[19] first suggested the use of sodium carbonate or bicarbonate in order to produce insoluble ferrous carbonate and later[20] advocated the use of aluminum hydroxide and bismuth sulfate.

Dimercaprol (BAL) was used despite the controversy over its toxicity. Peirce and Somers[2] report, after animal experiments, that the iron-BAL complex is more toxic than iron alone. However, the *British Medical Journal*[4] advocated its use "particularly where the metal has a characteristic sulfide." Murphy,[13] Roxburgh,[15] and Swift[22] used dimercaprol in their cases but did not evaluate it, whereas Shoss[16] thought it was a deciding factor in survival.

Murphy and associates[13] and Roxburgh[15] both reported positive urine sugar in their patients. It is our feeling that the two positive Benedict's reactions were due to nonspecific reducing substances and did not indicate hepatic, pancreatic, or renal damage with resultant abnormal carbohydrate metabolism or excretion as they were transient, and, in our patient, the blood sugar level was normal.

Spencer[21] reported serum iron determinations in two nonfatal cases (Table I, Cases 17 and 18) with results similiar to those recorded for this patient. Swift and associates[22] in their fatal case found a normal iron content in the liver and kidney. Smith[17] suggested that the intestinal barrier to iron is broken down by the initial large dose and raised the question that ferritin, acting as a vasodepressor substance, may cause the initial characteristic shock. He also suggested the possible value of using a phosphate salt for inhibition of iron absorption.

The *British Medical Journal*[5] states that, in all cases autopsied, there was a marked corrosive action on the gastric mucosa and less on the duodenum and small intestine. Prain[11] found definite necrosis and fatty metamorphosis in the liver and suggested that the absorbed iron was the toxic agent. Other authors and the JOURNAL[6] suggested that these changes are secondary to the shock resulting from the destruction of gastric mucosa.

Spencer[21] emphasized the characteristic picture of shock in four to six hours, improvement from twelve to thirty-six hours, and a second critical period from twenty to fifty-three hours. He also listed the classical

symptoms of pallor, coldness, tachycardia, retching, vomiting, drowsiness, and restlessness. He noted too that hematemesis was frequent, diarrhea uncommon, and the respiratory rate often rapid with shallow excursions.

This paper serves to re-emphasize the toxicity of ferrous salts and the necessity for rapid and conclusive treatment.

SUMMARY

1. A case of acute ferrous sulfate poisoning in a child with recovery is described.

2. A brief review of the literature is presented.

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THE COMPARATIVE BIOLOGICAL AVAILABILITIES OF FERROUS SULFATE IRON AND FERRIC ORTHOPHOSPHATE IRON IN ENRICHED BREAD[1]

HAROLD BLUMBERG AND AARON ARNOLD
Sterling-Winthrop Research Institute, Rensselaer, New York

THREE FIGURES

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According to the flour and bread enrichment program, as developed by federal and state agencies, the required iron should be added to the flour only in forms which are harmless and assimilable (Fed. Reg., '41). Primary consideration has naturally been given to the selection of iron preparations which exert no deleterious action upon the flour or bread. This technical requirement has somewhat obscured the basic nutritional motive for enrichment in that the degree of biological availability of the added iron has not been thoroughly considered. As has been pointed out by the Council on Foods and Nutrition of the American Medical Association ('41), obviously there is no nutritional advantage in adding an iron compound if this iron is not satisfactorily available to the body. Tobey and Cathcart ('41) pointed out that little information had been reported on the assimilability of iron compounds that were already in use for enrichment of flour.

Several forms of iron have been of practical interest for enrichment. The initial report on ferric phytate gave a somewhat favorable impression (Andrews, Evans and Huber, '41),

[1] Presented in part before the American Institute of Nutrition, Chicago, May 21, 1947 (Blumberg, H., and A. Arnold, '47, Fed. Proc., 6: 402).

but in subsequent rat tests (Nakamura and Mitchell, '43) and human studies (Moore, Minnich and Dubach, '43) it was found to be a comparatively poor source of iron. Little or no ferric phytate is now being used for flour enrichment. Sodium iron (ferric) pyrophosphate ($Fe_4(P_2O_7)_3 \cdot 2Na_4P_2O_7 \cdot 6H_2O$) was at first reported to be well utilized (Nakamura and Mitchell, '43), but in later investigations it was reported to have low availability (Street, '43; Freeman and Burrill, '45; Blumberg and Arnold, '47). Except for special products, sodium iron pyrophosphate is no longer being used to a great extent for enrichment of flour and bread. Reduced iron has been found to be highly available in rat investigations (Nakamura and Mitchell, '43; Freeman and Burrill, '45; Blumberg and Arnold, '47), as well as efficacious in clinical therapy (Fowler and Barer, '39). Reduced iron is now widely used in the mill enrichment of flour, as well as in other dietary products, such as infant foods and yeast.

Ferric orthophosphate has been the form of iron generally used when enrichment has been effected at the bakery. Day and Stein ('38) found ferric orthophosphate to be much less effective than ferric chloride for hemoglobin formation in rats. In their clinical studies on iron absorption, Moore and coworkers ('39) reported that the relatively insoluble ferric orthophosphate, as well as ferrous phosphate, was very poorly absorbed as compared with ferrous sulfate. In a single experiment on rats, Freeman and Burrill ('45) ranked ferric orthophosphate as only slightly less effective than the highly available ferric chloride. However, in 2 single-level experiments conducted as part of a comparative survey of iron sources, Blumberg and Arnold ('47) observed ferric orthophosphate to be less than one-half as effective as ferrous sulfate when the compounds were fed in enriched breads.

Ferrous sulfate is well known both experimentally and clinically as one of the most highly available forms of iron (Goodman and Gilman, '41). Recently ferrous sulfate has become of interest for bread enrichment.

In view of the large extent to which enrichment of the nation's bread is conducted at the bakery, it was considered desirable to obtain a better quantitative comparison of the relative biological availabilities of ferrous sulfate and ferric orthophosphate by testing each form of iron at several levels. The compounds were fed in the form of enriched breads containing the different iron sources, so that the iron would be tested in the same form as used for human consumption. A secondary comparison with ferric chloride was also made because some investigators have used the latter compound rather than ferrous sulfate as a standard of high biological availability.

EXPERIMENTAL METHODS

Diets

The diet employed in these experiments was similar to that previously used (Blumberg and Arnold, '47); the composition is given in table 1. Casein was included in the diet as a supplement to the inadequate protein of the bread, so as to provide sufficient protein for optimal hemoglobin regeneration. Low-iron casein, containing approximately 15 µg of iron per gm, was prepared in the laboratory from skimmed milk. The low-iron salt mixture was prepared by modifying U.S.P. XI Salt Mixture no. 2 in the following manner. The ferric citrate was omitted, of course. Since the bread supplied sodium chloride, this also was omitted from the mixture and the amount of salt mixture used was reduced from the usual 4% of the diet to 3%. Furthermore, potassium biphosphate was substituted for sodium phosphate.

Four lots of bread were baked from the same lot of flour with special enrichment mixes that supplied the usual amounts of thiamine, riboflavin, and niacin, but varied with respect to iron. One lot of bread was for the negative control and contained no added iron. The other 3 lots were enriched to provide approximately the following quantities of iron: (1) 131 µg/gm, as ferric orthophosphate; (2) 42 µg gm, as ferrous sulfate; and (3) 21 µg gm, as ferric chloride. The ferric orthophosphate was from a batch actually used for commercial

enrichment; the exsiccated ferrous sulfate was U.S.P. grade, and the ferric chloride was C.P. grade.

The breads were air-dried at 37°C. to a moisture content of approximately 4% and were then ground for use in the diets. Iron analyses of the breads were made by a thiocyanate procedure (Eckert and Auerbach, '44). The iron content of the negative control bread was found to be about 12 µg gm. The actual analyses indicated that the supplemented breads had slightly more than the intended additional iron contents, as follows: ferric orthophosphate 132 µg gm, ferrous sulfate

TABLE 1

Composition of diet.

BASAL MIXTURE		SUPPLEMENTS PER 100 gm BASAL MIXTURE [1]	
	%		mg
Bread (dried)	82	Thiamine hydrochloride	1
Casein (low-iron)	12	Riboflavin	2
Salt mixture (low-iron)	3	Pyridoxine hydrochloride	1
Corn oil	3	Calcium pantothenate	4
		Niacinamide (nicotinamide)	2
		Choline chloride	100
		Inositol	100
		Copper (as $CuSO_4 \cdot 5H_2O$)	3
		Manganese (as $MnSO_4 \cdot H_2O$)	1.5

[1] Each rat received by stomach tube a weekly fat-soluble vitamin supplement equivalent to 2000 U.S.P. units of vitamin A, 400 U.S.P. units of calciferol (vitamin D_2), and 10 mg of alpha-tocopherol.

43 µg/gm, and ferric chloride 26 µg gm. By suitable dilution with the negative control bread, the iron-enriched breads were made into the series of test bread mixtures shown in table 2. The 4 levels of ferric orthophosphate iron increased in geometric progression by multiples of 2.5, as follows: 8.4, 21.0, 52.5, and 131.2 µg/gm of bread, or 6.9, 17.2, 43.1, and 107.6 µg/gm of diet. The 4 levels of ferrous sulfate iron increased by multiples of 2, as follows: 5.25, 10.5, 21.0, and 42.0 µg/gm of bread, or 4.3, 8.6, 17.2, and 34.4 µg/gm of diet. The 2 levels of added ferric chloride iron were made the same as the intermediate levels of ferrous sulfate, i.e., 10.5 and 21.0 µg/gm of

bread or 8.6 and 17.2 μg/gm of diet, in order to permit comparison of these 2 highly available iron preparations.

The iron contents of the various diets are given in table 2. The extraneous, non-bread iron in the diets was only about 2 μg/gm. Diet 12 was prepared by addition of ferrous sulfate to the negative control diet 1 at a level of 244 μg of iron per gm of bread, or 200 μg/gm of diet. This provided a positive control to demonstrate the maximum rate of hemoglobin regeneration permitted by the basal diet in the presence of an amount of available iron known to be well within the optimal range.

TABLE 2

Iron contents of breads and diets.

GROUP	BREAD		NO. RATS	IRON CONTENT OF BREAD	IRON CONTENT OF DIET
	Compound added	Iron added			
		μg/gm		μg/gm	μg/gm
1	None (negative control)		9	12.0	11.9
2	Ferric orthophosphate	8.4	8	20.4	18.8
3	Ferric orthophosphate	21.0	8	33.0	29.2
4	Ferric orthophosphate	52.5	9	64.5	54.9
5	Ferric orthophosphate	131.2	10	143.0	118.0
6	Ferrous sulfate	5.25	9	17.2	16.2
7	Ferrous sulfate	10.5	9	22.5	20.5
8	Ferrous sulfate	21.0	10	33.0	29.2
9	Ferrous sulfate	42.0	9	54.0	45.0
10	Ferric chloride	10.5	9	22.5	20.5
11	Ferric chloride	21.0	10	33.0	29.2
12	Ferrous sulfate (positive control)	244.0	7	256.0	212.0

Animal experimentation

Albino rats of the Sherman strain were prepared for iron-deficiency studies by special feeding precautions generally similar to those described by Elvehjem and Kemmerer ('31). At about 25 days of age the weanling rats were removed to individual galvanized cages in which there was no exposed iron or rust. Anemia was induced by feeding certified cow's milk supplemented with cupric sulfate and manganous sul-

per liter. After the rats had been on the iron-depletion diet for 35 days, hemoglobin determinations were made on tail blood by the alkaline hematin method, as adapted for the Klett-Summerson colorimeter. Except for 12 somewhat resistant animals, the rats were found to be sufficiently anemic for test purposes, i.e., the hemoglobin values were 2.5–5 gm/100 ml, with an average of about 3.9 gm/100 ml.

The animals were divided into groups of 10 rats each, except for the positive control group 12, which had only 7 rats. Weight and sex distributions were similar in the various groups. Occasional mortality during the experiment reduced the numbers slightly, so that the final test groups contained 8–10 rats each, as shown in table 2. The experimental diets were then fed for 4 weeks, hemoglobin determinations and weighings being made at the end of each week. Groups 1, 2, 3, and 6, which were regenerating hemoglobin at slow rates, were continued on experiment for as long as 9 weeks for determination of the length of time required to reach a hemoglobin value of 10 gm/100 ml, i.e., close to the beginning of the normal range.

RESULTS

Ferrous sulfate and ferric orthophosphate

The general nature of the results of the comparison between ferrous sulfate and ferric orthophosphate is shown by the hemoglobin curves in figure 1. The rats fed the negative control diet and the 2 lower levels of ferric orthophosphate had not shown any increase in their hemoglobin concentration values by the end of the first week. However, since the values did increase during subsequent weeks, the readings at 1 week did not appear to offer a suitable basis for a valid comparison. On the other hand, some of the animals in the faster regenerating groups had already reached the normal range of hemoglobin values by the end of the second week. Consequently, the interpolated value for 1.5 weeks appeared to be the most sensitive point for comparison. When some later point on the

available forms may not be so marked or indeed may no longer be evident. With minor exceptions, the general trend of the results at 1.5 weeks is confirmed by the curves for other points during the test. The marked quantitative superiority of ferrous sulfate iron over ferric orthophosphate iron is readily apparent at the various levels of enrichment.

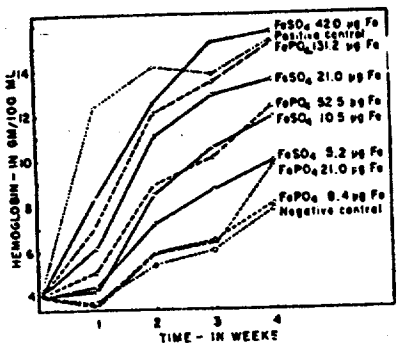

Figure 1

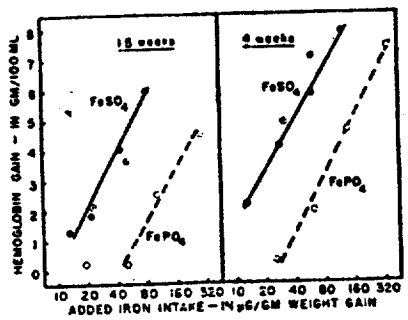

Figure 2

Fig. 1 Hemoglobin regeneration curves of anemic rats fed the indicated levels of ferrous sulfate iron (———) or ferric orthophosphate iron (– – – –), as supplied by enriched bread. Negative and positive control curves are also shown. All points were corrected to an initial hemoglobin value of 4.0 gm/100 ml of blood.

Fig. 2 Dosage response curves at 1.5 weeks and at 4 weeks of anemic rats fed ferrous sulfate iron or ferric orthophosphate iron, as supplied by enriched bread. The ferric chloride points are shown by the circled dots.

The initial hemoglobin values, as listed in table 3, show that the various test groups were at approximately the same degree of anemia at the start of the experiment. The mean hemoglobin gains and iron intakes after 1.5 weeks and 4 weeks are also given in table 3. These data were used to arrive at a definite figure for the comparative availability of ferrous sulfate and ferric orthophosphate in the following way. The total hemoglobin gains were corrected for the hemoglobin gain of the negative control (group 1), and the total iron intakes were corrected for the iron intake due to the diet itself without added iron (11.9 µg gm). This permitted calculation of hemoglobin gain per µg of added iron intake. The added iron intakes were also corrected for the minor differences in

TABLE 3

Responses of rats to various sources and levels of iron.

GROUP	DIET: Compound added	Iron added	NO. RATS	INITIAL WT. (AV.)	WT. GAIN (AV.) 1.5 wks.	WT. GAIN (AV.) 4 wks.	FOOD CONSUMPTION (AV.) 1.5 wks.	FOOD CONSUMPTION (AV.) 4 wks.	IRON INTAKE (AV.) 1.5 wks.	IRON INTAKE (AV.) 4 wks.	INITIAL HEMOGLOBIN (AV.)	HEMOGLOBIN GAIN MEAN ± S.E. 1.5 wks.	HEMOGLOBIN GAIN MEAN ± S.E. 4 wks.
		μg/gm		*gm*	*gm*	*gm*	*gm/day*	*gm/day*	*μg/day*	*μg/day*	*gm/100 ml*	*gm/100 ml*	*gm/100 ml*
1	None (negative control)		9	96	35	94	9.8	11.3	117	134	3.93	0.48 ± 0.26	3.69 ± 0.33
2	Ferric orthophosphate	8.4	8	84	36	90	9.4	12.3	177	231	3.90	0.69 ± 0.32	3.99 ± 0.44
3	Ferric orthophosphate	21.0	8	100	34	90	9.1	11.4	266	333	4.01	0.68 ± 0.33	5.64 ± 0.64
4	Ferric orthophosphate	52.5	9	92	45	111	10.5	12.9	576	708	3.86	2.96 ± 0.51	8.31 ± 0.57
5	Ferric orthophosphate	131.2	10	98	46	106	11.8	12.9	1389	1518	3.88	4.99 ± 0.76	11.11 ± 0.41
6	Ferrous sulfate	5.25	9	94	39	104	10.9	11.7	176	190	3.87	1.79 ± 0.14	5.84 ± 0.22
7	Ferrous sulfate	10.5	9	101	46	106	11.3	12.2	232	250	3.99	2.30 ± 0.37	7.82 ± 0.63
8	Ferrous sulfate	21.0	10	84	44	100	10.5	12.2	307	356	3.57	4.57 ± 0.39	9.48 ± 0.63
9	Ferrous sulfate	42.0	9	93	46	96	11.0	12.9	495	580	3.81	6.43 ± 0.28	11.62 ± 0.51
10	Ferric chloride	10.5	9	93	38	85	9.2	11.1	188	228	4.17	2.64 ± 0.45	8.62 ± 0.83
11	Ferric chloride	21.0	10	80	36	89	9.5	11.3	277	330	3.85	4.09 ± 0.29	10.77 ± 0.34
12	Ferrous sulfate (positive control)	244.0	7	84	45	101	8.7	11.1	1843	2352	3.39	9.23 ± 0.75	11.53 ± 1.8

the average weight gains of the groups, although such correction did not modify the final conclusions. This permitted calculation of hemoglobin gain per µg of added iron intake per gm of weight gain. The resultant dosage response values, illustrated graphically in figure 2, were then compared statistically by the method of Waddell and Kennedy ('47).

At 1.5 weeks ferric orthophosphate iron was 19.6 (± 2.4)% (mean ± S.E.) as available as ferrous sulfate iron if the lowest ferric orthophosphate value (group 2) is omitted from the calculation. This may be done on the grounds that the latter point appears to be below the sensitive portion of the dosage response curve. However, comparison of the 2 iron sources without omitting the group 2 value does not change the result markedly, though the standard error does not give so true a picture of the agreement of the data. Calculated to include all points at 1.5 weeks, ferric orthophosphate iron was 21.2 (± 6.8)% as available as ferrous sulfate iron.

At 4 weeks ferric orthophosphate iron was 25.2 (± 2.0)% as available as ferrous sulfate iron. As indicated previously, the latter time does not really give a valid comparison, but it has been included to demonstrate the marked differences in availability which exist even at this less sensitive point.

The excellent agreement between the groups fed differing amounts of the same added iron source is demonstrated in figure 2. Aside from the 1.5-week value for group 2, as noted above, the points fall very nearly on straight lines.

The statistical significance of the differences between the individual groups was determined by calculation of the standard error of the difference between the mean hemoglobin gains both at 1.5 weeks and at 4 weeks, comparisons being made for all groups. A relatively high criterion of significance was adopted by basing conclusions only on P values of 0.01 or less (i.e., probability of the difference being fortuitous equals 1 in 100, or less). The results of this analysis showed that the previously mentioned differences in mean hemoglobin gains between the ferric orthophosphate and ferrous sulfate groups

($FeSO_4$ iron 10.5 µg/gm) was significantly superior (P = 0.005) to group 3 ($FePO_4$ iron 21 µg/gm), and was not significantly different (P = 0.3) from group 4 ($FePO_4$ iron 52.5 µg/gm). Likewise, group 9 ($FeSO_4$ iron 42 µg/gm) was greatly superior (P = < 0.001) to group 4 ($FePO_4$ iron 52.5 µg/gm), although not significantly superior (P = 0.09) to group 5 ($FePO_4$ iron 131.2 µg/gm). The results of the analysis at 4 weeks were almost as significant.

TABLE 4

Rates of hemoglobin regeneration, based upon days required to reach hemoglobin value of 10 gm per 100 ml.

GROUP	BREAD		DAYS	RATE OF HEMOGLOBIN REGENERATION AS PERCENTAGE OF OPTIMUM
	Compound added	Iron added		
		µg/gm		
1	None (negative control)	..	63.0	0
2	Ferric orthophosphate	8.4	47.4	3
3	Ferric orthophosphate	21.0	37.8	6
4	Ferric orthophosphate	52.5	20.5	18
5	Ferric orthophosphate	131.2	10.7	42
6	Ferrous sulfate	5.25	28.7	11
7	Ferrous sulfate	10.5	18.9	21
8	Ferrous sulfate	21.0	12.4	36
9	Ferrous sulfate	42.0	9.8	47
10	Ferric chloride	10.5	20.1	19
11	Ferric chloride	21.0	13.3	33
12	Ferrous sulfate (positive control)	244.0	5.4	100

The results were also calculated in terms of the number of days required to reach an average hemoglobin value of 10 gm 100 ml, which is close to the normal range (see table 4). The various groups were then rated for percentage of optimal hemoglobin regeneration by comparison with the positive control, group 12, which contained a known excess of ferrous sulfate. A correction was made for the hemoglobin regeneration contributed by the iron present in the diet without iron enrichment (group 1, negative control bread). By this method

fate iron was approximately 4 to 6 times as effective as ferric orthophosphate iron. Statistical treatment of these data by the previously mentioned procedure (Waddell and Kennedy, '47) showed ferric orthophosphate iron to be 17.2 (± 6.2)% as available as ferrous sulfate iron. This is in general agreement with the comparison based on hemoglobin regeneration at 1.5 weeks.

As shown in table 3, all of the groups of rats grew well. The weight gains of the various groups differed little and thus were in marked contrast to the wide variations in hemoglobin regeneration. Likewise, the differences in food consumption were small, corresponding generally to the small differences in weight gains, and could not account for the large differences in hemoglobin regeneration. In addition, several ferrous sulfate rats were pair-fed with ferric orthophosphate rats to maintain the same individual food consumption. The ferrous sulfate animals again proved much superior in hemoglobin regeneration. A comparison of iron intakes and hemoglobin gains (see table 3) emphasizes the superiority of the biological availability of ferrous sulfate iron over that of ferric orthophosphate iron.

Ferrous sulfate and ferric chloride

The comparison of ferrous sulfate and ferric chloride at 2 levels showed these forms of iron to be equally effective for regeneration of hemoglobin. The curves for the 2 compounds are practically superimposable, as may be seen in figure 3. Further evidence of similarity is presented by the hemoglobin gains in table 3 and the hemoglobin regeneration rates in table 4. Calculations were made for the standard error of the difference between the mean hemoglobin gains. The analysis at 1.5 weeks showed that there was no significant difference ($P = 0.56$) between group 7 ($FeSO_4$ iron 10.5 µg /gm) and group 10 ($FeCl_3$ iron 10.5 µg /gm). Similarly, there was no significant difference ($P = 0.32$) between group 8 ($FeSO_4$ iron 21 µg /gm) and group 11 ($FeCl_3$ iron 21 µg /gm). The analysis

difference between the ferrous sulfate and ferric chloride values. The highly available ferric chloride iron, like that of ferrous sulfate, was 4 to 5 times as effective as ferric orthophosphate iron (see fig. 2). This study indicated that results based on ferric chloride standards should be directly comparable with those based on ferrous sulfate standards.

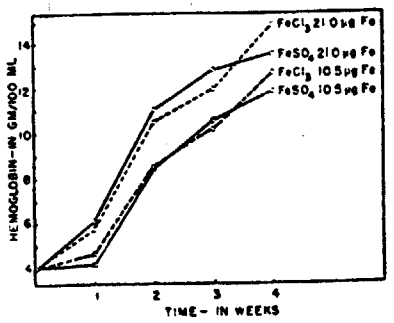

Fig. 3 Hemoglobin regeneration curves of anemic rats fed similar levels of ferrous sulfate iron (———) and ferric chloride iron (– – – –), as supplied by enriched bread. All points were corrected to an initial hemoglobin value of 4.0 gm/100 ml of blood.

DISCUSSION

Several experiments have now been reported on the utilization of ferric orthophosphate iron in rats. Although Day and Stein ('38) did not attempt a truly quantitative comparison, their results in a single-level study indicated that ferric chloride iron was approximately 4 times as available as ferric orthophosphate iron. In their single experiment, Freeman and Burrill ('45) found ferric orthophosphate iron to be practically as effective as ferric chloride iron. Inasmuch as Freeman and Burrill report only the final hemoglobin values at the end of 28 days, a time at which the groups were already well within the normal hemoglobin range, it is possible that differences occurring at 1 to 2 weeks were no longer apparent. In 2 previous single-level experiments, Blumberg and Arnold ('47) found ferrous sulfate iron to be more than twice as available as ferric orthophosphate iron. The present investigation at

conditions of these experiments, ferrous sulfate iron or ferric chloride iron is approximately 4 to 5 times as available for hemoglobin regeneration as is ferric orthophosphate iron. It may be noted that McCance et al. ('43) observed in human subjects that the addition of disodium phosphate to bread decreased the absorption of iron. Caution must be exercised against confusing ferric orthophosphate itself with preparations in which the ferric orthophosphate has been solubilized with sodium citrate, e.g., Soluble Ferric Phosphate, N. F. VIII (National Formulary, '46).

The magnitude of the enrichment program justifies a thorough appraisal of the assimilability of the iron sources in use or proposed for use. Although the results of rat experiments are not applicable to man with certainty, these investigations with enriched bread strongly suggest the advisability of seeking better sources of iron than ferric orthophosphate for bread enrichment. The clinical findings of Moore and coworkers ('39) suggest that in man also ferric orthophosphate is poorly utilized as compared with ferrous sulfate. Certainly further studies upon the efficacy of ferric orthophosphate in both animals and man should be conducted if its use is to be continued. From a nutritional standpoint it would appear safer to use an iron source already known to be highly efficacious in man, such as ferrous sulfate, reduced iron, or other preparations of comparable availability. In order that the consumer may secure the full benefit of the enrichment program, it is desirable that highly assimilable forms of iron be used in bread and flour enrichment.

SUMMARY

The biological availabilities of the iron in ferrous sulfate and ferric orthophosphate have been compared on the basis of hemoglobin regeneration in rats made anemic from iron deficiency. A secondary comparison was made with ferric chloride. The iron compounds were fed in the form of enriched breads, and multiple levels of iron enrichment were

Under the conditions of these experiments, ferrous sulfate iron was 4 to 5 times as available as ferric orthophosphate iron when both compounds were tested at 4 widely spaced levels.

When compared at 2 levels, ferric chloride iron was equal in biological availability to the highly effective ferrous sulfate iron, or 4 to 5 times as available as ferric orthophosphate iron.

Attention is called again to the desirability of using highly assimilable forms of iron in flour and bread enrichment, so that the consumer may secure the full benefit of the enrichment program.

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FERROUS SULFATE POISONING

Report of a Fatal Case

By LIEUT. LEROY K. BRANCH, M.C., U.S.N.R.
Key West, Fla.

FERROUS sulfate has been considered a nontoxic drug, regardless of the amount ingested. Medicinal iron is not mentioned as a possible cause of fatal poisoning in textbooks of pediatrics or pharmacology.

The purpose of this paper is to report a case of ferrous sulfate poisoning with fatal outcome in a 29 month old child.

REVIEW OF THE LITERATURE

Only a few cases of iron toxicity were reported before 1947. These were reviewed by Smith, Jones and Cochran[1] in 1950.

Veeder[2] reviewed 8 fatal and 8 nonfatal cases of poisoning from accidental ingestion of medicinal iron reported since 1947. Smith's editorial[3] pointed out the importance of knowing the potential toxicity of ferrous sulfate and other medicaments containing iron.

Forbes[4] initiated current interest in this problem by reporting two fatal cases of ferrous sulfate poisoning in 1947. Later by experimentation he established the lethal dose of ferrous sulfate in cats as 0.065 gm./64 gm. body weight. Thompson[5, 6] reported two fatal human cases in 1947. He recorded four other cases in 1950, one of which was fatal. Foucar et al.[7] reported a 26 year old man who died three hours after ingestion of one-fourth pound of ferrous sulfate. Roxburgh[8] made no claim for the value of 2,3 dimercaptopropanol (BAL) used in the treatment of a 16 month old patient who recovered from iron poisoning. Lindquist[9] reported a nonfatal case of ingestion of ferric chloride tablets and mentioned a nonfatal case of Magnussen's which had not been reported. Smith, Jones and Cochran[1] described a case in a 17 month old child with autopsy findings.

Murphy and his associates[10] recorded the recovery of a 30 month old child after ingestion of 15 gm. ferrous sulfate (1.28 gm./kg. body weight). Spencer[11] described eight cases of iron poisoning in the British literature. Four of these were fatal. The number of reported cases of iron poisoning in children totals 25 since 1947 with the recent description of one fatal and two nonfatal cases by Duffy and Diehl[12] and a fatal case by Swift and associates.[13] Twelve of the 25, as well as the one case in an adult, ended fatally.

CASE REPORT

D. J., a 29 mo. old white male, was found eating ferrous sulfate tablets. He was seen by another physician, who lavaged the stomach within ½ hr. after ingestion of the tablets. Patient was allowed to go home when a normal physical condition was determined. Physician did not know what amount of drug had been ingested.

From the Department of Pediatrics, U. S. Naval Hospital, Key West, Fla.

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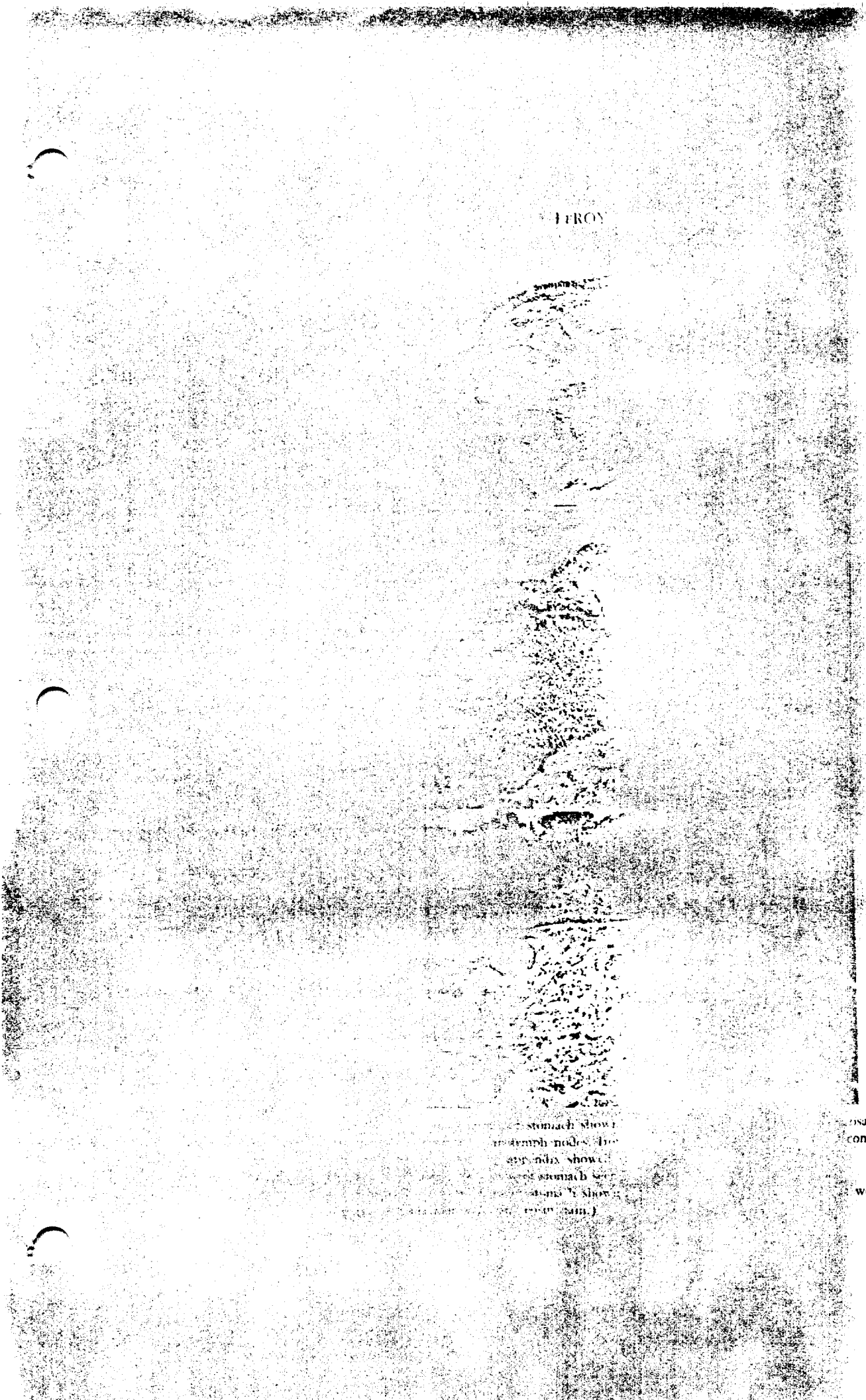

...stomach show...
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...osa (iron stain).
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...well as necrosis

After the child returned home, vomiting, hematemesis and loose black stools appeared. Cyanosis was noticed about 1 hr. later. At time of admission to hospital (3½ hr. after ingestion of tablets) he was comatose and appeared moribund. There was no response to painful stimuli and no reflexes were present. Mucous membranes and nail beds were intensely cyanotic. Widely dilated pupils responded very sluggishly to light and corneal reflexes were absent. Respirations were fast, shallow and irregular. Heart beat was faint and irregular. The abdomen showed moderate distention, but no palpable organs or masses. Large brownish-black tarry stools were passed during examination.

Oxygen, external warmth, suction, intravenous fluids and blood were given as soon as possible. Death occurred 1 hr. after admission, 4½ hr. after ingestion of iron tablets. Blood studies for methemoglobin as well as other laboratory data were not obtained due to presence of extreme shock. Postmortem toxicologic studies showed no poisonous agents other than iron.

Recheck of history indicated that more than 60 and possibly up to 75 ferrous sulfate tablets (1.87 gm./kg. body weight) had been ingested. These 0.3 gm. tablets contained no other active ingredients.

Autopsy Findings*

Body was that of a well developed, and well nourished boy weighing 13.5 kg., and measuring 92 cm. in length. Cyanosis of head, shoulders, fingertips and toes was intense. Petechial hemorrhages were present over head, neck and chest. Edema of neck and generalized serous effusions were present. Marked pulmonary edema without infarction was present. Right side of heart showed acute dilatation. Hemorrhagic, necrotizing gastroenteritis involved stomach, small and large intestine, and even portions of appendix. Submucosal venous thromboses with iron pigment deposition were demonstrated in numerous areas of stomach. (See Figs. 1-3.)

Discussion

The history presented here of accidental ingestion of large amounts of iron by a small child is typical of most previous case reports. Characteristic vomiting, hematemesis, frequent tarry stools, vasomotor collapse, and cyanosis were present in this case. Part of the pathologic picture of pulmonary edema, acute dilatation of the right side of the heart, and edema of the neck may have been caused by intravenous fluids administered near and after death. The finding of hemorrhagic gastroenteritis with necrosis and slough has been recorded in most previous reports of iron poisoning. Gastric submucosal venous thromboses with iron pigment were demonstrated in this case.

It seems strange that a long and widely used drug such as ferrous sulfate had not been incriminated as an important poison until 1947. It is likely that other cases have been unrecognized or unreported.

Prevention of poisoning is paramount in therapy. The public, as well as all physicians, should be aware of the potential danger from ingestion of medication containing iron.

Thompson[5,6] found that emesis may rid the stomach of iron tablets even as late as one hour after ingestion. He believed that gastric lavage with bicarbonate solution converted the iron to the less irritating ferrous carbonate and also diluted the poison. Possibly feeding of raw eggs, milk or bismuth preparations would help protect the mucosa. Edge and Somers[4] found in work done on mice BAL-iron combinations to be more toxic than iron alone. Treatment of vascular collapse with blood, plasma and other fluid is important. The use of oxygen and other supportive measures is helpful.

Summary

A fatal case of ferrous sulfate poisoning in a 20 month old boy with autopsy findings is recorded. He died approximately 4½ hours after ingestion of about seventy-five 0.3 gm. ferrous sulfate tablets (1.87 gm./kg. body weight). Signs of severe gastrointestinal

* Autopsy performed by Lieutenant Commander C. G. Bratenahl, MC, USN.

irritation were followed by cyanosis and peripheral vascular collapse. Hemorrhagic gastroenteritis with mucosal slough and submucosal venous thromboses were demonstrated post mortem. Prevention and measures for correction of shock were emphasized in discussion of treatment. This case report should re-emphasize the potential danger of iron poisoning.

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SPANISH ABSTRACT

Relato de un Caso Fatal de Intoxicación por Sulfato Ferroso

Se presenta un caso fatal de intoxicación por ingestión accidental de sulfato ferroso en un niño de 29 meses de edad. El enfermo murió a las 4½ horas aproximadamente después de la ingestión de cerca de 75 tabletas de 0.30 gramos de sulfato ferroso (1.87 gramos por kilo de peso corporal). Presentó síntomas de irritación gastrointestinal severa seguidos de cianosis y colapso vascular periférico, encontrándose histopatológicamente gastroenteritis hemorrágica con esfacelo de la mucosa y trombosis venosas submucosas.

Se enfatiza en el tratamiento el uso de sangre, plasma, líquidos, oxígeno, etc. para el colapso vascular y el peligro potencial de la intoxicación por hierro, debiendo prevenirse al público de esta eventualidad.

U. S. Naval Hospital

Acta Med. Scand.
171 Suppl. 376: 7-22, 1962

FROM THE DEPARTMENT OF MEDICINE II (HEAD: PROFESSOR ERIK WASSÉN), UNIVERSITY OF GÖTEBORG, SAHLGRENSKA SJUKHUSET, GÖTEBORG, SWEDEN.

A METHOD FOR COMPARATIVE STUDIES ON IRON ABSORPTION IN MAN USING TWO RADIOIRON ISOTOPES

By

HANS BRISE AND LEIF HALLBERG

INTRODUCTION

The amount of iron absorbed at one occasion can be accurately determined using available radioiron methods[1, 2, 3]. In comparative studies (e.g. the absorbability of different iron compounds) other methodological problems will arise. When comparing the absorption of iron in two groups of individuals treated in different ways, the great variation in absorption between individuals will make the results from such studies very difficult to interprete even when using accurate methods to determine the absorption. Because of this, comparisons have often been made in the same subject. However, great variation in the absorption of iron occurs not only between individuals but also within a single individual on different days. Both these sources of variation were considered when the present method for comparative studies on iron absorption was designed.

The method was based on a repeated administration of iron to each individual (one dose on each of 10 days), giving on alternate days two iron compounds, each compound labelled with a different radioiron isotope (Fe^{55} or Fe^{59}). Determinations of Fe^{55} and Fe^{59} activities were made in a blood sample drawn 2 weeks after the last oral iron dose, when an optimal utilization of absorbed iron for hemoglobin synthesis could be expected to have taken place[4–7]. From these determinations the relative absorbability of the two compounds could be calculated. By giving repeated iron doses (5 doses of each compound on alternate days) the error due to the variation in absorption on different days could be reduced by more than half, and by making the comparisons within the same subject the variation in absorption between individuals was eliminated.

In 1958 a preliminary report was given on the method[8]. In the present paper the details of the experimental procedure are given and the validity of the method is more thoroughly tested. As an example of the application of the method, a study of the relative absorbability of ferrous- and ferric iron is included.

MATERIAL

Sixtytwo subjects were included in this study. One subject (1-M-T) had a hypernephroma without demonstrable metastasis. One subject (26-M-BII) had a Biliroth II gastric resection several years ago. Three subjects had an iron deficiency anemia after acute blood loss (ID). The other subjects were healthy volunteers (N), some of whom had served as blood donors (BD). In the tables (M) denotes male and (F) female subjects.

PRINCIPLE OF METHOD AND EXPERIMENTAL PROCEDURE

The experimental design is outlined in figure 1. Unless otherwise stated, the same amount of elemental iron was given every morning for 10 days after an overnight fast. The iron was labelled with Fe^{55} and Fe^{59} on alternate days. When comparing ferric and ferrous iron, for instance, the compounds were labelled with different radioiron isotopes and were given on alternate days. To reduce systematic errors the first iron dose was alternately labelled with Fe^{55} and Fe^{59}, and was also alternately ferrous and ferric iron. From analysis of Fe^{55} and Fe^{59} activity in a blood sample drawn 2 weeks after the last oral dose the mean absorption ratio was calculated.

Each subject received a box containing 10 consecutively numbered 25 ml flasks [illegible] were taken in order. Detailed written and oral instructions were given for the experiment.

The iron solution was taken directly from the flask. This was then filled with tap water and the rinse water was also taken. This procedure was repeated so that the total volume consumed was 75 ml. No food or drink was taken for an additional two hours.

The residual radioactivity in the flask was less than 0.5 per cent of the original content. This determination was made using a scintillation detector with a 5 inch. × 6 inch. plastic crystal with a well to contain the whole flask.

ORAL IRON DOSES

The ferrous iron in this study was $FeSO_4 \cdot 7\ H_2O$ (Merck, pro analysi). The ferric iron content was less than 1 per cent as found by analysis using the thiocyanate method[10].

The ferric iron salt administered was $Fe_2\ (SO_4)_3\ 6\ H_2O$ (Union Chimique Belge pour analyse).

The volume of the solution in each flask was 25 ml and contained 30 mg of elemental iron, 10 mg of ascorbic acid (to prevent oxidation of ferrous iron — no ascorbic acid was added to ferric iron solutions), 4 gram of sucrose and 4 [illegible] of radioiron. In the solutions containing 5 mg of iron the ascorbic acid content was reduced proportionally to 1.2.3 mg

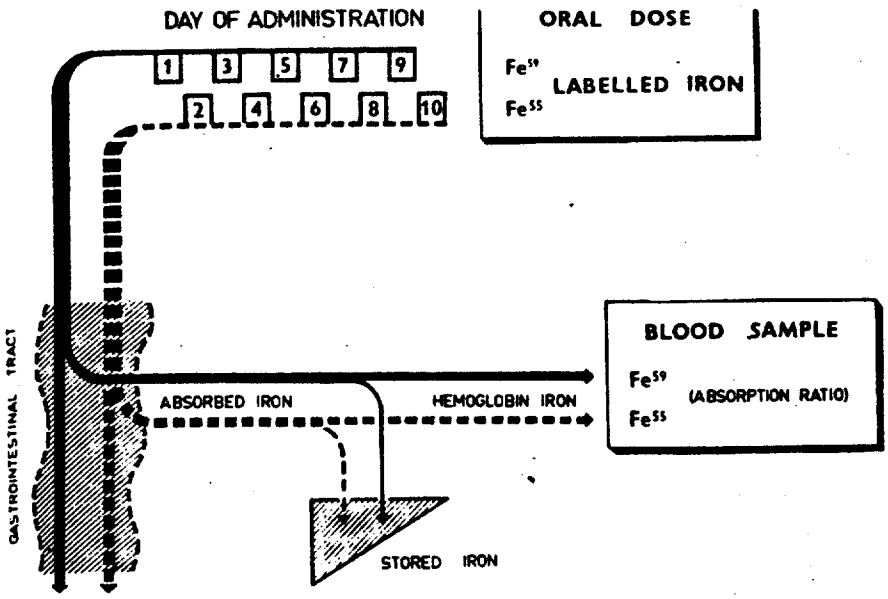


Fig. 1. Experimental design.

freshly boiled distilled water was used in preparation of the solutions and nitrogen was bubbled through the solutions in flasks before closing. The ferric iron content in the ferrous sulphate solutions these 25 ml flasks was less than 1 per cent after 4–6 weeks storage in room temperature.

The Fe^{55} and Fe^{59} was obtained from Abbott Laboratories, Oak Ridge, Tennessee U.S.A. as a solution of $FeCl_3$ (pH less than 1.5). The specific activity of Fe^{59} was 5–13 microcuries per microgram and the specific activity of Fe^{55} was 2–3 microcuries per microgram respectively. Stock solutions of Fe^{55} and Fe^{59} in 0.02 N HCl containing 2–3 μC of radioiron per ml were prepared from the original solutions. The final pH in the administered solutions was 2.5. The total amount of radioactivity administered to each subject was less than 25 μC Fe^{59} and 25 μC Fe^{55}.

As ferric chloride was used to label the ferrous sulphate the isotope exchange was tested. An acid solution containing the two compounds was transferred to a separatory funnel and extracted with isopropylether, which will extract only ferric ions under the conditions used[9]. A complete exchange was found to have taken place as the radioactivity in the isopropylether layer was less than 2 per cent of the original amount.

RADIOACTIVE ANALYSIS

From the blood sample (150 ml), drawn in 50 ml ACD-solution[1]) 2 weeks after the last oral iron dose, four samples each

[1]) 1.2 g of sodium citrate, 0.48 g of citric acid, 1.47 g of glucose to 100 ml with water.

containing 5 mg of iron were digested, the iron electroplated and the Fe^{55} and Fe^{59} activity determined as previously described[11]. The mean values obtained were used.

Standard solutions were prepared from the described stock radioiron solutions. Two milliliters of stock solution were transferred 4 times to a 1 000 ml measuring flask using the same pipette as that used in preparing the oral doses. The flasks were then filled up with 0.02 N HCl.

A known amount of the standard solution was digested with inert iron (to give 5 mg of iron) and electroplated together with the unknown samples. From each standard solution 6 electroplated reference samples were made. The unknown samples were measured together with the reference samples in an automatic sample changer.

CALCULATIONS

The Fe^{55} and Fe^{59} activities per 5 mg of iron in circulating red cells were determined according to formulas given in an earlier paper[11].

The amount of absorbed iron labelled with Fe^{55} or with Fe^{59} in circulating red cells was calculated from the activities of Fe^{55} and Fe^{59} in the administered doses, and from the activities in the blood according to the following equation:

$$\frac{a \cdot D}{100} = F \cdot TFe \quad \ldots\ldots\ldots\ 1$$

where

a = per cent of the administered iron in the circulating hemoglobin mass

D = administered radioactivity (Fe^{55} or Fe^{59}).

F = observed radioactivity (Fe^{55} or Fe^{59}) per milligram of iron in blood

TFe = total amount of circulating hemoglobin iron in milligrams

TFe was calculated from the estimated blood volume (males = weight in kilograms × 74.2; females = weight in kilograms × 65)[26], the hemoglobin concentration in the blood and with the presumption that 1 g hemoglobin contains 3.34 milligrams of iron accordingly. Hemoglobin was determined as cyanmethemoglobin[12].

$$TFe = \frac{BV \cdot Hb \cdot 3.34}{100} \quad \ldots\ldots\ 2$$

where

BV = estimated blood volume in milliliters

Hb = hemoglobin concentration in grams per 100 ml blood.

When two compounds were compared in this experimental design a figure of the relative absorbability of the two compounds was obtained according to the following equations:

$$A = \frac{a}{k} \quad \ldots\ldots\ldots\ 3$$

where

A = total amount of absorbed iron in per cent of the amount administered

k = fraction of absorbed iron in the circulating hemoglobin mass

a was calculated from Equation 1.

and,

Absorption ratio:

$$\frac{A_{55}}{A_{59}} = \frac{F_{55} \cdot D_{59} \cdot k_{59}}{F_{59} \cdot D_{55} \cdot k_{55}} \quad \ldots\ldots 4$$

where

A_{55} and A_{59} = total amount of absorbed iron labelled with Fe^{55} and Fe^{59} respectively in per cent of the amount of iron administered,

and,

D_{55} and D_{59} = total amount of administered Fe^{55} and Fe^{59} respectively.

Assuming that the difference of the average internal distribution of absorbed iron on different days is negligible (i.e. $k_{59} = k_{55}$) the absorption ratio can be calculated from the simplified equation.

$$\text{Absorption ratio} = \frac{A_{55}}{A_{59}} = \frac{F_{55} \cdot D_{59}}{F_{59} \cdot D_{55}} \quad \ldots 5$$

It is obvious that the accuracy of the estimation of TFe does not influence the accuracy of the absorption ratio. The figures for "Absorption" given in the tables are calculated from the estimates of TFe. Because of this fact these figures are not true expressions for the total absorption since only the absorbed iron utilized for red cell formation is included. The "Absorption" figures are given only to facilitate comparisons between individuals.

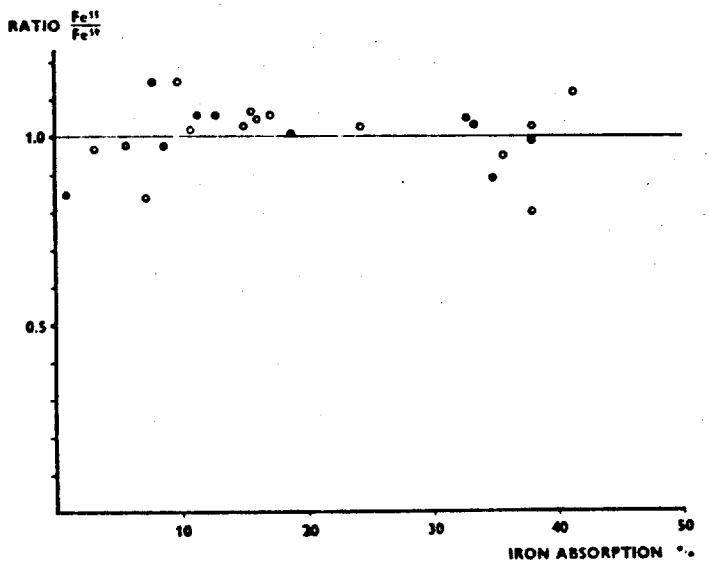


Fig. 2. Precision and accuracy of method. Absorption ratio of Fe^{55} labelled and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days. (Each dose was equivalent to 30 mg of elemental iron.) Observed absorption ratio values were plotted against estimated absorption. Results in subjects starting with Fe^{55} were indicated as dots ●, in those starting with Fe^{59} as rings ○.

CONTROL STUDIES

Even when the foregoing experimental design is used, the accuracy of comparisons of the absorbability of different iron compounds is limited by (a) the day to day variation in the absorption of iron and (b) the variation of the internal distribution of the absorbed iron to erythropoiesis and storage. In order to be able to calculate the magnitude of the total variation the following studies were made.

TABLE 1

Precision and accuracy of method. Administration of Fe^{55} and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days. Each dose equivalent to 30 mg of elemental iron.

SUBJECT	First dose	"ABSORPTION" (per cent)		ABSORPTION RATIO Fe^{55} Fe^{59}	
		Fe^{55}	Fe^{59}	Individual value	Mean value
1-M-T	Fe^{55}	0.7	0.9	0.86	
2-M-N	Fe^{55}	8.7	7.8	1.11	
3-M-BD	Fe^{55}	8.5	8.7	0.98	
4-M-N	Fe^{55}	12.1	11.4	1.06	
5-M-BD	Fe^{55}	13.6	12.8	1.06	
6-M-BD	Fe^{55}	18.9	18.8	1.01	
7-M-BD	Fe^{55}	34.2	32.7	1.05	
8-M-BD	Fe^{55}	34.2	33.3	1.03	
9-F-BD	Fe^{55}	30.8	34.8	0.89	
10-M-BD	Fe^{55}	37.5	37.9	0.99	1.00
11-M-N	Fe^{59}	3.1	3.2	0.97	
12-M-N	Fe^{59}	5.6	5.7	0.98	
13-M-N	Fe^{59}	6.5	7.7	0.84	
14-F-N	Fe^{59}	11.3	9.8	1.15	
15-M-BD	Fe^{59}	11.1	10.8	1.02	
16-M-BD	Fe^{59}	15.1	15.0	1.03	
17-F-BD	Fe^{59}	16.7	15.6	1.07	
18-F-BD	Fe^{59}	16.8	16.1	1.05	
19-M-BD	Fe^{59}	18.1	17.1	1.06	
20-M-BD	Fe^{59}	25.1	24.1	1.03	
21-F-N	Fe^{59}	33.9	35.6	0.95	
22-M-BD	Fe^{59}	30.2	37.9	0.80	
23-M-BD	Fe^{59}	29.1	37.9	1.03	
24-M-BD	Fe^{59}	46.1	41.2	1.12	1.01

Absorption ratio: Mean value: 1.01
Standard error of mean: ±0.02
Standard error: ±0.09

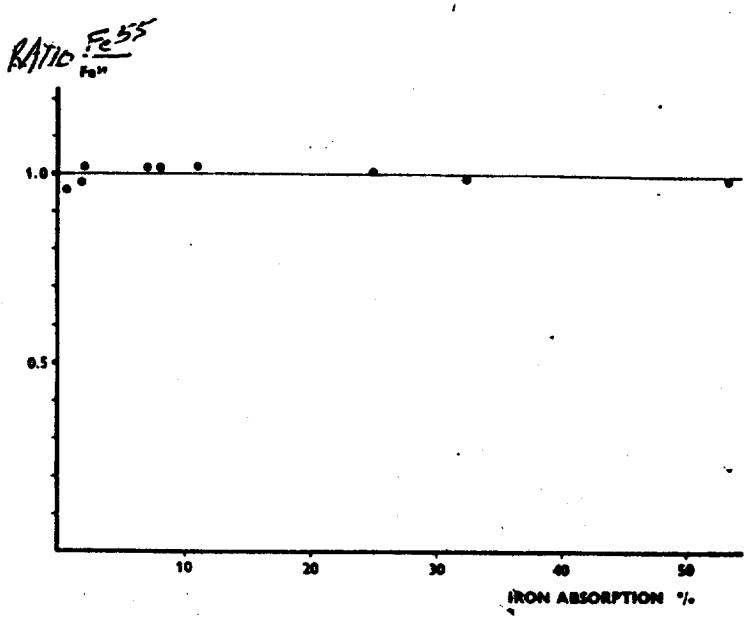


Fig. 3. Analysis of experimental error of method. Absorption ratio of Fe^{55} and Fe^{59} labelled iron from a single dose (30 mg Fe) containing known amounts of both isotopes. Results plotted against estimated absorption.

Ferrous sulphate was given as a solution containing 30 mg of elemental iron on each of 10 days labelled with Fe^{55} or Fe^{59} respectively on alternate days. Ten subjects started with Fe^{55} labelled iron and fourteen subjects with Fe^{59} labelled iron.

The obtained results are given in table I and graphed in figure 2. The mean value of the absorption ratio (Fe^{55} Fe^{59} labelled iron) in those subjects starting with Fe^{55} labelled iron was the same as in those starting with Fe^{59} labelled iron (1.00 and 1.01 respectively).

To be able to calculate that part of the variation which is due to a varying absorption and internal distribution of iron on different days, the experimental error was calculated in the following way:

Nine subjects were given one 30 mg iron dose of ferrous sulphate labelled with known amounts of both Fe^{55} and Fe^{59}. A blood sample was drawn 2 weeks later and the absorption ratio was calculated as described previously. The results are given in table II and in figure 3.

The standard error was ± 2.2 per cent. Because the ratio Fe^{55} Fe^{59} must be the same in the blood sample as in the dose administered in this experimental design the calculated standard error must be identical with the experimental error of the method.

This experimental error does not include the variation of emptying and rinsing of the flasks containing the iron doses. However, this latter error is quite negligible

tivity in the flasks (less than 0.5 per cent).

From the figures obtained for the total variation S_t; (Variance = 0.008017, S = ± 0.09 — see table I) and experimental error S_{exp}; (Variance = 0.000487, S = ± 0.02 — see table II) it is possible to calculate the sum of the real variation in absorption and internal distribution of absorbed iron (S_a), using the following formula for resolution of a variance in two components.

$$S_t^2 = S_{exp}^2 + S_a^2$$

The calculated real variation in the absorption and distribution of absorbed iron was thus found to be ±9 per cent — variance 0.007530. This means that the experimental error only forms a negligible part of the total variation.

Analysis of experimental error of method. Administration of a single iron dose (30 mg Fe) containing known amounts of Fe^{55} and Fe^{59}.

SUBJECT	"ABSORPTION" (per cent)		ABSORP. TION RATIO
	Fe^{55}	Fe^{59}	Fe^{55}/Fe^{59}
25-M-N	0.7	0.7	0.96
26-M-B II	1.8	1.9	0.98
27-M-N	2.1	2.1	1.02
28-M-N	7.2	7.1	1.02
29-F-N	8.2	8.1	1.02
30-F-N	11.3	11.1	1.02
31-F-ID	25.3	25.0	1.01
32-F-ID	32.3	32.5	0.99
33-M-ID	53.0	53.5	0.99

Absorption ratio: Mean value: 1.00
Standard error of mean: ±0.01
Standard error: ±0.02

TABLE III

Precision and accuracy of method. Administration of Fe^{55} and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days. Each dose equivalent to 5 mg of elemental iron.

SUBJECT	First dose	"ABSORPTION" (per cent)		ABSORPTION RATIO Fe^{55}/Fe^{59}	
		Fe^{55}	Fe^{59}	Individual value	Mean value
34-M-N	Fe^{55}	7.6	7.6	1.01	
35-M-N	Fe^{55}	9.1	7.8	1.17	
36-M-N	Fe^{55}	9.9	9.0	1.10	
37-M-BD	Fe^{55}	19.9	17.8	1.12	
38-M-BD	Fe^{55}	37.5	35.7	1.05	1.09
39-M-N	Fe^{59}	13.0	10.5	1.23	
40-F-N	Fe^{59}	19.8	17.3	1.14	
41-M-BD	Fe^{59}	27.3	32.1	0.85	
42-F-BD	Fe^{59}	47.0	61.6	0.76	
43-M-BD	Fe^{59}	66.0	78.7	0.84	0.96

Absorption ratio: Mean value: 1.0
Standard error of mean: ±0.0
Standard error: ±0.1

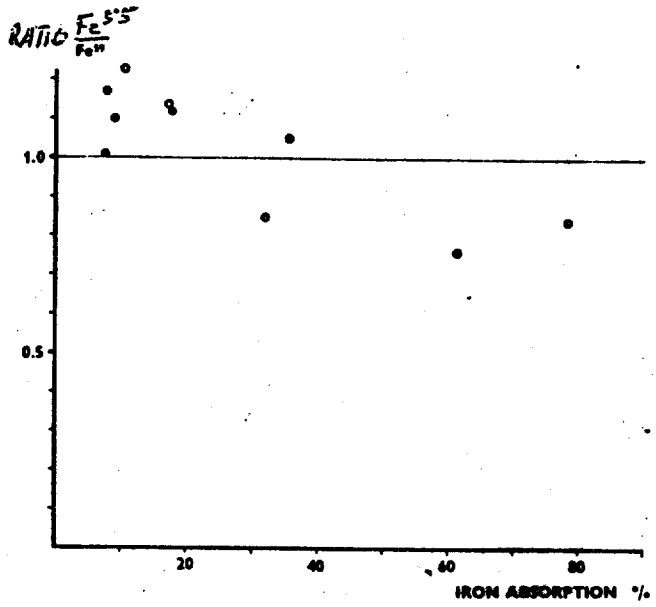


Fig. 4. Precision and accuracy of method. Absorption ratio of Fe^{55} labelled and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days. (Each dose was equivalent to 5 mg of elemental iron.) Observed absorption ratio values were plotted against estimated absorption. Results in subjects starting with Fe^{55} were indicated as dots •, in those starting with Fe^{59} as rings ○.

As S_a is obtained from a mean value of 5 pairs of comparisons the variation in absorption and utilization of absorbed iron from one day to another within the single individual can be calculated as $\sqrt{5 \times 0.007530} = \pm 20$ per cent (coefficient of variation).

The variation in absorption on different days was also studied when using 5 mg doses, because such a dose is more closely related to physiological conditions and has been recommended as the most satisfactory dose for testing iron absorption[13]. In this series comprising 10 subjects the 5 mg iron dose was given for 10 days in the same way as in the first series. The results obtained are given in table III and figure 4.

The observed standard deviation of the absorption ratio was 16 per cent. By resolution of the variance in the two components as above the variation in absorption on different days within the single individual using 5 mg doses was $\sqrt{5 \times 0.024794} = \pm 35$ per cent (coefficient of variation). As found by an F — test the standard deviation was greater when 5 mg doses were used than when 30 mg doses were used ($p < 0.05$).

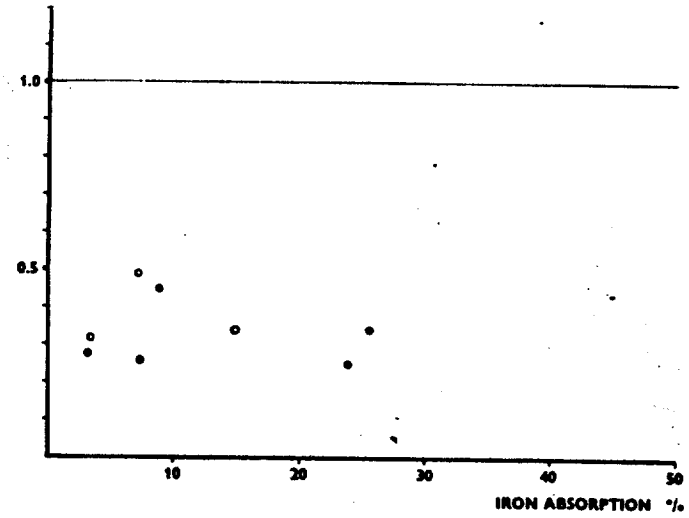


Fig. 5. Absorption of ferric versus ferrous iron at different estimated absorption levels. Each iron dose was equivalent to 40 mg of elemental iron. (• indicate subjects starting with ferrous sulphate, ○ indicate subjects starting with ferric sulphate.)

COMPARISON OF THE ABSORPTION OF IRON FROM FERROUS AND FERRIC SULPHATE

As an example of the application of this double isotope method a comparison of the absorption of iron from ferrous and ferric sulphate is included in the present paper.

It has repeatedly been shown, using different methods that ferrous iron is more readily absorbed than ferric iron[11–17]. Because of this a comparison of ferrous and ferric iron may also serve as an indirect check of the method. The data on the quantitative importance of the valency of iron are greatly diverging. The present method can be expected to give more exact information on the long debated problem.

a. Oral iron dose 30 mg.

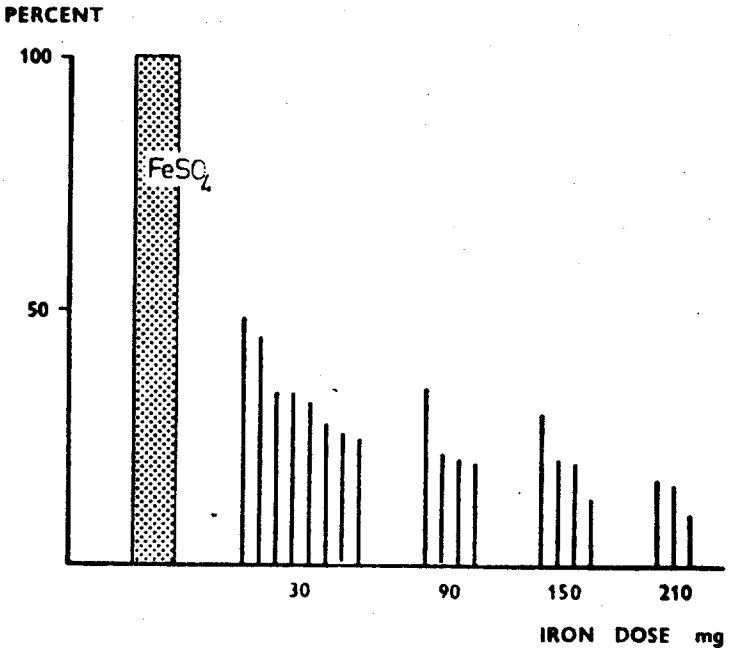
Eight subjects were included in this study. The solutions were prepared as previously described (ascorbic acid was not added to the ferric sulphate solutions) and each dose contained 30 mg of elemental iron. In five subjects the ferrous iron was labelled with Fe^{59}, in three subjects with Fe^{55}. In order to further reduce the possibility of systematic errors in this comparison 5 subjects started with the ferrous dose and 3 subjects with the ferric dose.

The results are given in table IV and are illustrated in figure 5. In the figure the absorption ratio ferric/ferrous iron is graphed against the absorption of iron from the ferrous sulphate solution. The term "absorption" is used to mean the

Absorbability of ferric and ferrous sulphate. (Each dose equivalent to 30 mg of elemental iron.)

SUBJECT	First dose	"ABSORPTION" (per cent)		ABSORPTION RATIO Ferric Ferrous iron
		Ferric iron	Ferrous iron	
44-M-N	Ferrous iron[59]	0.9	3.2	0.28
45-M-N	Ferric iron[55]	1.1	3.4	0.32
46-M-BD	Ferric iron[55]	3.5	7.2	0.49
47-M-BD	Ferrous iron[59]	1.9	7.4	0.26
48-F-BD	Ferrous iron[59]	4.0	8.9	0.45
49-M-BD	Ferric iron[52]	5.2	15.0	0.34
50-M-BD	Ferrous iron[55]	6.0	24.0	0.25
51-M-BD	Ferrous iron[55]	8.7	25.7	0.34

Absorption ratio: Mean value: 0.34
Standard error of mean: ±0.03
Standard error: ±0.09



6. Absorption of ferrous and ferric iron at different dosage levels. The same amount of iron given each day. Each line represents absorption of ferric iron as a percentage of the absorption of ferrous iron in the same subject.

per cent of absorbed iron in circulating red cells 2 weeks after the administration of the last oral iron dose.

The mean value of the absorption ratios in these 8 subjects was 0.34 ± 0.03 and it is thus quite clear that ferrous iron is much more readily absorbed than ferric iron.

b. Oral iron dose 90—210 mg.

It is possible that the magnitude of the iron dose may influence the relative absorbability of ferrous and ferric iron. An additional study was thus made in which ferrous and ferric iron were compared at higher dose levels (90, 150 and 210 mg of elemental iron). The same amounts of ferrous and ferric iron were thus given to each subject.

The results are given in table V and are also graphed in figure 6 where each bar represents one subject. It is evident that the greater absorbability of ferrous iron was more pronounced the higher the iron dose. A correlation analysis between absorption ratio and iron dose gave the following results: $r = -0.50$ and $p < 0.05$.

When 30 mg iron doses were compared, 3 times more ferrous iron was absorbed. When 90, 150 and 210 mg doses of iron were studied, respectively 4, 5 and 7 times more ferrous than ferric iron was absorbed.

TABLE V

Absorbability of ferric and ferrous sulphate at different dose levels (90—210 mg).

Daily oral dose (mg Fe)	SUBJECT	First dose	"ABSORPTION" (per cent)		ABSORPTION RATIO Ferric/Ferrous iron	
			Ferric iron	Ferrous iron	Individual value	Mean value
90	52-M-BD	Ferric	1.0	3.0	0.35	
90	53-M-BD	Ferric	1.6	7.5	0.22	
90	54-M-BD	Ferrous	2.7	12.8	0.21	
90	55-F-BD	Ferrous	3.1	16.6	0.20	0.25
150	56-M-BD	Ferrous	1.3	6.1	0.21	
150	57-M-BD	Ferric	1.0	7.7	0.13	
150	58-M-BD	Ferric	1.7	8.5	0.20	
150	59-M-BD	Ferrous	7.5	25.1	0.30	0.21
210	60-M-BD	Ferrous	0.5	4.4	0.10	
210	61-M-BD	Ferrous	1.0	6.1	0.17	
210	62-M-BD	Ferric	1.2	7.5	0.16	0.14

COMMENT

This method was devised in an attempt (a) to make more valid comparisons of the absorption of iron from different iron compounds and (b) to facilitate the quantitation of factors influencing the absorption of iron. An example of the latter application of the method is a study of the effect of meals on iron absorption presented in a preliminary report[8].

Earlier comparative studies have almost exclusively been devoted to the relative absorbability of different iron compounds [18–24]. The comparisons have usually been based on determinations of the regeneration rate of hemoglobin during iron therapy in iron deficient subjects. Two or more groups of subjects treated with different iron compounds have been compared. However, there are numerous factors influencing the therapeutic response to iron (severity of anemia, continued bleeding, condition of iron stores, infections etc.) which often make such comparisons difficult to interpret and necessitate comparisons between large homogenous materials.

Using the method described in this paper, the main sources of error in comparative studies on iron absorption are greatly diminished. The repeated administration of iron reduces the average variation in absorption and internal distribution of absorbed iron within the single individual to less than one half. Since the absorption ratio is a mean value of five pairs of comparisons, inasmuch as the single subject serves as his own control valid conclusions can be drawn from materials containing relatively few individuals. For the same reason the requirements of a selection and classification of subjects for comparative iron absorption studies are also markedly reduced.

The method is convenient since it is not necessary to quantitate the total absorption (e.g. by faeces collection) to be able to study the effect of a substance on iron absorption or the relative absorbability of iron from two compounds.

Iron doses labelled with different isotopes were not given on the same day in order to diminish the possibility of an exchange of radioiron between different doses in that part of the intestine where a measurable absorption could take place.

The effect of a preceding dose on the absorption of a subsequent dose was found to be negligible in this experimental design since the mean value of the obtained absorption ratio (Fe^{55} labelled ferrous sulphate Fe^{59} labelled ferrous sulphate) in the group starting with one isotope did not differ from the mean value in the group starting with the other isotope.

The 30 mg iron dose was most thoroughly studied because it can be considered to be a therapeutic oral dose. The 5 mg iron dose was also studied inasmuch as it may represent an optimal physiological iron dose. The observed greater variation in the absorption of this small dose, may be explained by the relatively greater influence of extraneous random factors (e.g. adsorption to mucin or protein components in the gastrointestinal tract).

The sources of error in this method are of two kinds. One kind consist in analytical errors and errors in the administration of the iron doses. The magnitude of these

errors was found to be only about 2.5 per cent. The other and main source of error is the variation in absorption and distribution of absorbed iron. This error can be further reduced only by giving more iron doses for longer time.

In 10 subjects a blood sample was drawn not only 2 weeks after the last oral dose but also after 3 weeks and in 5 of the subjects at times up to 2 months after the last dose. The difference between the absorption ratios within the single subject was of the same magnitude as the experimental error. The effect of a variation in internal distribution of absorbed iron on the real absorption ratio can be expected to decrease in time. The fact that no significant difference between absorption ratios was found, when followed for longer time indicates that the main part of the observed total variation is related to a variation in absorption from day to day. This variation was about ± 20 per cent when 30 mg iron doses were given and about ± 35 per cent when 5 mg doses were given. This great variation means that it is very difficult or impossible to demonstrate minor differences in absorbability of two compounds, if such a comparison is based on determinations of iron absorption on two occasions in the same subject, even if these determinations are made with a very accurate method. The great variation in absorption of iron on different days in the same subject stresses the importance of giving iron in repeated doses in comparative studies in the same individual.

The degree of underestimation of the real absorption, as calculated from the radioactivity in the red cell mass 2 weeks after the last oral dose, does not influence the absorption ratio. These "absorption figures" have only been given as a rough classification of the subjects' avidity to absorb iron.

The observed lower absorption of ferric iron (compared with ferrous) is consistent with earlier observations[14–17]. From the observed difference in absorbability it is not necessary to postulate that iron is absorbed only in the ferrous state. The difference can most easily be explained from the well known physico-chemical difference between ferric and ferrous ions. At the pH existing in the gastrointestinal tract, a considerably greater amount of the ferric than of the ferrous iron will be present as undissociated hydroxide. Moreover ferric iron has a greater avidity to form insoluble compounds or complex compounds than ferrous iron. The average ionic concentration of iron in the upper part of the intestinal tract, where the absorption of iron mainly takes place, can thus be expected to be much higher when ferrous iron is given than when ferric iron is given.

The difference in relative absorbability between ferrous and ferric iron can be expected to be more pronounced the higher the iron dose because at higher dose levels the ferric ion concentration will remain constant while more and more undissociated ferric hydroxide will be formed.

This reasoning is consistent with the observed decrease of the ferric/ferrous iron absorption ratio with increased iron doses (0.31 : 0.11 at the dose levels 30 and 210 mg of iron respectively).

It is also consistent with the observation by BONNET, HAGEDORN and OWEN who found no difference in absorbability of ferrous and ferric iron when very small amounts (50 μg) of elemental iron were

used[25]. At the much lower concentration of iron achieved in the gastrointestinal tract with this extremely small iron dose, it can be expected that ferrous and ferric iron will both be present in ionic form to the same degree (the solubility product of ferric hydroxide will not be exceeded).

From the present studies it can be concluded that considerably more iron is absorbed from ferrous than from ferric sulphate. At therapeutic dose levels (30 mg of iron or more) at least 3 times more iron will be absorbed if given in the ferrous state. This difference in absorbability between ferrous and ferric iron is of such a magnitude that it can be concluded that ferric iron has no place in oral iron therapy.

SUMMARY

A method is described which is especially devised for comparative studies of the absorbability of different iron compounds and for a quantitation of the influence of various factors on iron absorption.

Two radioiron isotopes are used — Fe^{55} and Fe^{59}. One iron compound is labelled with one isotope and one compound with the other. The compounds (and isotopes) are administered on alternate days for ten consecutive days.

From analysis of Fe^{55} and Fe^{59} in one blood sample drawn two weeks after the last oral dose the relative absorbability of different iron compounds can be determined.

By giving ferrous sulphate labelled with the two isotopes on alternate days the accuracy and precision of the method has been determined. The average day to day variation in absorption of iron in the single individual was found to be about ± 20 per cent using 30 mg doses and ± 35 per cent using 5 mg doses.

As an example of the application of the method the absorbability of iron from ferrous and ferric sulphate has been studied at different dosage levels. It was found that about 3—7 times more iron was absorbed from ferrous sulphate than from ferric sulphate.

The results show that the method will greatly facilitate comparative iron absorption studies since each subject serves as his own control.

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THE MECHANISM OF ACUTE FERROUS SULPHATE POISONING

R. J. K. BROWN, M.B., B.Chir.(Cantab.), M.R.C.P., D.C.H.(Lond.),* *London, Eng.*
and J. D. GRAY, M.D.(McGill),† *Halifax, N.S.*

IN SPITE OF repeated warnings, acute iron poisoning in childhood is still of much too frequent occurrence. On reading the relevant literature it is plain that no entirely satisfactory explanation of its mechanism has as yet been advanced; its treatment must therefore remain empirical until more is known.

We record a case showing some hitherto unpublished facts. The speculations which followed the clinical observations have led to some experimental work, the outcome of which is also embodied in this paper.

Clinical.—At 4 p.m. on October 5, 1953, a 2-year-old boy swallowed 40 ferrous sulphate tablets. About 5 minutes later his mother returned to the room and saw what had happened. He was given a Seidlitz powder, after which he vomited a brown-stained fluid; very shortly thereafter he slid into semi-coma. At 4.35 p.m. he was admitted to King's College Hospital where his stomach was washed out with a 25% sodii bicarbonate solution; this returned a dark brown fluid with fragments of tablets. A few ounces of solution were left in the stomach.

One hour and fifteen minutes after ingestion he was extremely cold and pale with slight cyanosis of the extremities and an almost imperceptible pulse at the wrist. The respirations were rapid and shallow. In three hours and fifty minutes his general condition had improved, pulse 140 per minute, volume poor. B.P. 105/70, liver edge soft and at the costal margin. He had vomited small quantities of bright blood and passed copious soft black stool. Straight radiograph of the abdomen showed no radio-opaque material in the stomach or duodenum. Intravenous infusion of 5% glucose in 1/5 N. saline was started.

Up to about 19 hours after ingestion he continued to vomit mucus and bright red blood. He passed two more copious black stools. Urinary chlorides were absent. The infusion was then changed to normal saline, followed by two pints of casein hydrolysate and compound vitamin B preparation.

Nineteen hours: now rational, vomited coffee-ground substance. He developed a loose cough and was pyrexial; chest examination, however, revealed no moist sounds.

Twenty-five hours: urine contained bilirubin, acetone, reducing substance and chlorides, but no albumin; oral methionine and vitamin E therapy begun.

Thirty-one hours: his condition was deteriorating, he was drowsy and restless and had a high-pitched cry. Pulse and respiratory rates were increased, reflexes depressed. Plantar response flexor.

Forty-two hours: he was by now deeply comatose, the breathing stertorous. He had an occasional mild convulsion. Slight jaundice was noted and the liver was now one finger-breadth below the costal margin. His face was puffy and suggested œdema; abdominal reflexes were absent; the plantar responses were now extensor. His urine had the "raw meat" smell of amino acids. Bilirubin was present. Paper chromatography confirmed the abundance of urinary amino acids. He was given intramuscular paraldehyde to control the fits.

Forty-six hours: convulsions still occurring. The pulse was rapid and feeble, the respirations were irregular and shallow. One gram of potassium was added to the intravenous solution (see biochemical findings). The bed was tilted head down and the nasopharynx sucked out. There were palpable and audible coarse rhonchi in the chest. He was now obviously jaundiced. The liver edge was firm and 1½ finger-breadths below the costal margin. Glutamic acid therapy was begun; 250,000 units of penicillin 6-hourly was also started.

Fifty-six hours: when seen at this time he was in a quiet deep sleep with regular respirations. Considerable œdema was present, the jaundice was deepening. The liver was still enlarged. Glutamic acid treatment was continued.

Seventy hours: still rather drowsy, pulse full and bounding; B.P. 130/50. The urine was less dark and the amino acid output decreased.

Four days: he was mentally normal but very weak. The jaundice was still present. There was pitting œdema of the ankles and sacrum. The chest was radiographically normal. The liver was firm and felt two finger-breadths below the costal margin.

Seven days: the general improvement had continued although the liver was still enlarged. Intravenous therapy was discontinued. From now on it became obvious that he was developing pyloric stenosis; a month later, this complication was relieved by gastroenterostomy. At the time of operation a piece of liver was taken for biopsy. Histological examination of the section showed normal architecture with slight increase in the reticuloendothelial cells which contained some iron pigment.

Biochemical findings.—Forty-two hours after ingestion his serum sodium, plasma chlorides, alkali reserve, blood urea, blood sugar, serum calcium and alkaline phosphatase were all within normal limits. The cerebrospinal fluid was normal. Serum potassium was 9.2 mgm. % (2.4 mEq./litre). Direct van den Bergh positive. Bilirubin 6.5 mgm %; at the end of 7 days this had dropped to 2 mgm. %. Four days after ingestion the urinary iron output over 24 hours was 2.8 mgm. % (normal 0.5-1 mgm.). The serum iron was 5.45 mgm. % three hours, 430 µgm. 42 hours, 153 µgm. 56 hours, 57 µgm. 4 days and 149 µgm. 7 days after ingestion. Combining power was nil after 4 days and 75 after 7 days. The changes in plasma proteins are shown in Table I.

Experimental methods and materials. — 1.5 gm. of analar grade ferrous sulphate was dissolved in 10 ml. sterile distilled water; this solution was kept sealed under partial vacuum until required, in order to prevent the oxidation of the ferrous salt. It was made up about two hours before use. Rabbits were the experimental animals. Ten were selected weighing between 1.6 and 2.2 kgm. and 0.5 ml. of the ferrous sulphate solution containing 75 mgm. of the salt was injected into the marginal ear vein of each animal. A further three were chosen as controls; 0.5 ml. of sterile distilled water was given by the same route to each. Four of the test animals died within 24 hours, one was killed 24 hours after injection, two at 2, one at 3, one at 6 and one at 10 days. The controls were killed 1 day, 2 days and 3 days after the administration of water. Brain, liver, spleen and kidneys were removed from all animals after death, fixed in 5% formol saline and stained by hæmatoxylin and eosin and the Prussian blue reaction for histological examination. Plasma proteins were estimated by the micro-Kjeldahl method and identified by paper chromatography; plasma amino acid nitrogen by the method of Dapielson.

*Children's Department, King's College Hospital, and the Belgrave Hospital for Children, London, England.
†Pathological Department, Halifax Infirmary, Halifax, N.S.

TABLE I.

THE EFFECT OF ACUTE FERROUS SULPHATE POISONING ON PLASMA PROTEIN

Time after ingestion	*Turbidity tests*	*Serum Proteins* albumin gm. %	globulin gm. %	*Electrophoretic pattern*	*Ppt. of protein with tungstic acid*
42 hours	Clear solution	3.11	0.84	?Decrease of globulin	Normal cloud
56 hours		5.24*	0.24	No proteins detected	No precipitation
7 days	1 unit	3.40	0.95	Normal pattern	Normal cloud
15 days	1 unit			?Additional band	Normal cloud

*As no protein bands were detected on electrophoresis and tungstic acid precipitation did not occur, these figures are interpreted as representing non-protein nitrogen.

Results.—Ferrous sulphate ($Fe_2 SO_4 . 7 H_2O$) has a molecular weight of 278. The atomic weight of iron, being 55.8, is equivalent to one-fifth the weight of the salt; therefore 75 mgm. of ferrous sulphate, the dosage used in these experiments, would contain 15.5 mgm. of Fe ++. The actual amount given in mgm. per kgm. body weight and its effect on the survival rate are shown in Table II.

TABLE II.

THE INTRAVENOUS DOSAGE OF FERROUS SULPHATE AND IRON IN MGM. PER KGM. AND ITS EFFECT ON SURVIVAL RATE

Rabbit No.	$Fe_2 SO_4 7 H_2O$ *mgm. per kgm.*	Fe ++ *mgm. per kgm.*	*Survival and time*
1	47.0	9.4	8 hours
2	47.5	9.5	16 "
3	47.0	9.4	16 "
4	46.0	9.3	24 "
5	44.0	8.8	Killed at 24 hours
6	40.0	8.0	" " 48 "
7	40.0	8.0	" " 48 "
8	37.0	7.4	" " 72 "
9	36.0	7.3	" " 6 days
10	35.0	7.0	" " 10 "

The minimum lethal dose of ferrous sulphate appears to be about 46 mgm. per kgm. or 9.3 mgm. of Fe ++ per kgm. for rabbits. The amount required to bring about death within 24 hours is critical, for it will be noted that rabbit No. 5 survived this length of time on a dose of ferrous sulphate that was only 2 mgm., or 0.5 mgm. Fe ++ per kgm. less than that which killed the preceding animal. Using guinea pigs, Edge and Somers[1] found 6.1 mgm. of Fe ++ per kgm. was the lethal quantity, which accords reasonably well with our findings when species difference is taken into account.

The immediate effect of the introduction of ferrous sulphate into the animal circulation was striking. It has previously been described by Somers.[2] A few seconds after the injection was completed the animals were prostrated, lying on their stomachs with head lolling to one side, the hind legs outstretched; the respiratory rate increased; the bladder and the bowels emptied; and occasional short, sharp contractions of the hind limbs were seen. After about 15 minutes a partial recovery set in. It was noted that those animals still very ill when returned to the animal house subsequently died within 24 hours.

In seven experimental animals and in the three controls the plasma proteins and plasma amino acid nitrogen were measured. In 4 experimental and 1 control the partition of globulins was examined by paper chromatography. The effect of intravenous ferrous sulphate on these is shown in Table III. The histological changes found in the organs of the experimental animals were as follows:

Liver.—In death occurring under 12 hours: the lobular pattern is normal. The portal vessels and most of the sinusoids are filled with blood, the latter most marked at the lobule periphery. The parenchymal cells are œdematous, the cytoplasm foamy, the nucleus normal. The Kupffer cells, particularly those nearest the portal areas, contain iron; there is little to be seen in the cells of the parenchyma (Fig. 1). From 12-24 hours: patchy variations in parenchyma cell size appear. In some areas the cells are so œdematous that there is a concomitant sinusoidal obliteration, in others there has been some cell shrinkage. The vascular tree is still engorged. The cytoplasm of the parenchymal cells now shows vacuolation. The nucleus is still normal. There is a heavy concentration of iron in the Kupffer cells appearing as minute dots with a perinuclear distribution; the iron has also appeared in some of the parenchymal cells, again with a perinuclear distribution. The deposit of iron within the lobules is variable. In some it extends from the portal areas to the central vein, in others a third to half a lobule is affected. The spread, however, seems to be peripheral to central, for whenever part of a lobule is affected it is nearly always peripheral.

From 24-48 hours: large areas of coagulation necrosis are present. The nuclei in the parenchymal cells of the

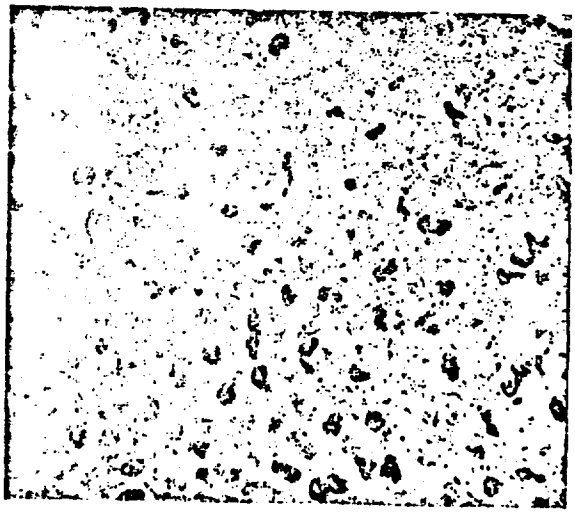

Fig. 1.—Section from the liver of a rabbit dying 8 hours after an intravenous dose of ferrous sulphate. The Kupffer cells are loaded with iron; the parenchymal cells, relatively free. Prussian blue reaction x 360.

TABLE III.

THE EFFECT OF INTRAVENOUS FERROUS SULPHATE ON PLASMA PROTEINS, PLASMA AMINO ACID NITROGEN, AND GLOBULIN PARTITION IN RABBITS

	Plasma proteins gm. %			*Globulin partition*			
Time of death after dose	*Total*	*Albumin*	*Globulin*	*Amino acid nitrogen mgm. %*	*γ*	*α*	*β*
24 hours and under	5.1	4.88	0.22	—	+	0	0
1							
2	3.78	3.78	0	18.5	—	—	—
48 hours							
1	5.3	3.50	1.8	10.2	+	+	0
2	4.41	2.67	1.74	9.0	+	+	0
3 days	6.51	4.60	1.91	8.1	—	—	—
6 days	5.1	3.5	1.60	9.9	+	+	+
10 days	6.54	4.17	2.37	4.2	—	—	—
Controls							
1	6.7	4.44	2.26	7.8	+	+	+
2	6.83	3.50	3.33	6.6	—	—	—
3	6.25	3.7	2.55	7.0	—	—	—

The presence of a specific globulin on the paper strip is indicated in the table by + and its absence by 0.

necrotic parts show pyknosis and fragmentation. Vascular engorgement has reached its maximum. The iron has moved out of the Kupffer cells, for they are now relatively free of its deposit. Parenchymal cells show the metal in two forms. In some its presence is indicated by multiple blue dots, in others—and these appear to be the majority—as a diffuse bluish cytoplasmic "smoke" with no evidence of droplet formation. Some of the canaliculi contain iron "thrombi."

At 48 hours coagulation necrosis has increased in extent, and there is a moderate infiltration of eosinophils along many of the remaining sinusoids. Most of the iron has been removed, for only an occasional cell gives a positive Prussian blue reaction. At the third day the histological picture shows little change from that described at 48 hours.

At six days there is no apparent difference between the livers of control and test animals histologically.

Spleen.—In death under 12 hours: the splenic sinuses are so packed with red blood cells as to give the lymphoendothelial tissue an appearance of islands in a sea of blood. There is no evidence of necrosis. Scattered phagocytes are filled with iron granules. From 12-24 hours: the hæmorrhage is still present. Lying in an occasional relatively unobscured sinus is a clear bluish fluid whose characteristics suggest an iron bound protein-complex. At 48 hours: the hæmorrhage has abated, the normal splenic pattern is returning. The protein-iron-complex

Fig. 2

Fig. 3

Fig. 2.—Section from the spleen of a control rabbit, showing few scattered deposits of iron. Prussian blue reaction x 90. Fig. 3.—Section from the spleen of a rabbit killed 10 days after an intravenous dose of ferrous sulphate, showing the storage of unwanted metal in the organ. Prussian blue reaction x 360.

is not present, and there appears to be a considerable deposition of iron in the sinus lining cells.

From the third day onward the spleens of the control and test animals are in general similar, but the amount of iron stored in the tissue histiocytes appears greater in test animals than that shown in controls. This impression is confirmed when the spleen of the animal killed 10 days after the administration of ferrous sulphate is compared with that of the untreated one. It would seem that from the third day onward, iron which has not been excreted is gradually "buried" in the spleen (Figs. 2 and 3).

Kidneys.—In death under 12 hours: the glomerular capillaries are dilated and engorged with red blood cells. The interstitial vessels are congested. There is no evidence of iron in any part of the nephron. From 12-24 hours: the vascular congestion has increased. The epithelium shows cloudy swelling. Between the glomerular tuft and the capsule, the same protein-iron-complex seen in the splenic sinuses is present; it can also be found in the lumina of some of the proximal and distal convoluted tubules (Fig. 4). From 24-48 hours: the vascular congestion is much less marked. Cloudy swelling of the epithelium is still present. There is a colourless protein in the lumina of the straight tubules. From the third day onward there is no essential difference between the test and control kidneys.

Brain.—No alteration in the normal histological pattern could be found in any sections examined during the period of observation.

Lung.—In death under 12 hours: the pulmonary vessels and alveolar capillaries are stuffed with red blood cells. There is considerable œdema of the alveolar walls. The alveoli themselves are free of exudate. There are patchy areas of lung collapse. Some of the intracapillary phagocytes contain iron droplets. From 24-48 hours: there is a marked lessening in the vascular congestion. Œdema of the alveolar walls is still present and some of the alveoli contain an acellular exudate. A few histiocytes give a positive Prussian blue reaction. By the third day the lung approximates the normal.

A comparison between the clinical findings and experimental results is shown below.

The patient poisoned himself by absorbing unknown quantities of iron from the gut, whereas the rabbits were poisoned by a single intravenous dose. In the experiment, artificial continuity is obtained by building a composite picture from the effects of the metal in a series of rabbits with a variable time factor; yet there is surprising agreement between the findings in each.

During a ten-year period up to the end of 1953 there were 11 patients admitted to King's College Hospital or the Belgrave Hospital with iron poisoning. In one, the poisonous substance was ferrous gluconate; in the remainder, compound tablets of ferrous sulphate. There were no deaths, but in three patients the illness was severe and in one it was critical (the case described in this paper). In one other patient definite hepatic enlargement was noted at 36 hours though no anxiety was felt about her general condition. Serum iron in this patient was 872 μgm. per 100 ml. after three hours. The complication of pyloric stenosis has been observed twice in this series.

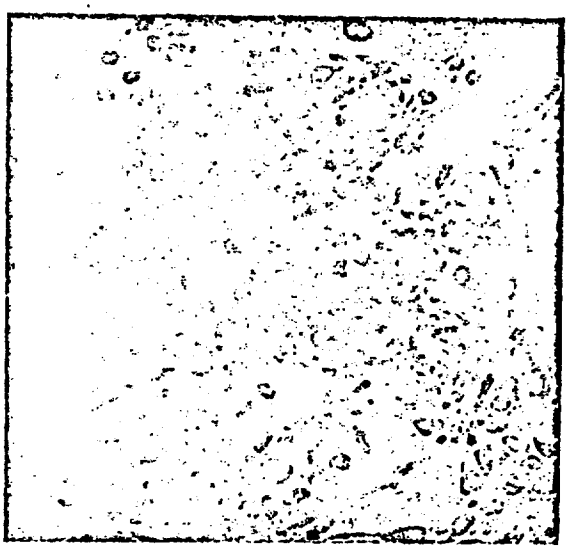

Fig. 4.—Section from the kidney of a rabbit dying 20 hours after a dose of ferrous sulphate intravenously. A protein-iron-complex is filling the glomerular vascular tree. Prussian blue reaction x 360.

DISCUSSION

The movement of iron from the gut to the tissue of utilization is a complex procedure. The rate of dissociation of iron salts is equated to acidity; therefore absorption takes place, for all practical purposes, in the stomach and upper small bowel. Entrance into the receptor cells of the mucosa is dependent on the presence of apoferritin; if the available concentration has already been converted to ferritin, iron absorption is blocked until the metal in the gut mucosa can be transferred to the plasma. It is moved in the latter in the form of ferric beta globulin; in turn, plasma iron can only be stored in tissue as ferritin. Iron may be essential for life but the

Time after poisoning	*Clinical*	*Experimental*
0 - 12 hours	Period of shock. Liver not enlarged.	Period of shock. Marked reduction in plasma globulin. Histological changes in liver slight.
12 - 24 hours	Apparent improvement. Evidence of liver damage.	Necrosis of liver. Rise of plasma amino acids.
24 - 48 hours	Condition deteriorating. Reduction in plasma globulin. Liver enlarged. Jaundice present.	Necrosis of liver. Plasma globulin reduced. Beta globulin absent. Plasma amino acids raised.
48 - 56 hours	Comatose. Liver enlarged. Jaundice increased. No protein detected in plasma.	Necrosis of liver. Plasma globulin low. Beta globulin not present. Plasma amino acids raised.
72 hours	Improvement apparent. Liver function returning.	Liver damage still evident. Plasma globulins still low and amino acids raised.
7 days	Improvement continuous. Liver still enlarged. Plasma globulins low.	Histologically, liver appears normal. Plasma globulins low. Globulin partition normal. Plasma amino acids still above normal.

body appears to treat it as a potentially dangerous criminal in need of constant guard, for at no time during the process of absorption, movement and utilization is it allowed to roam in a free ionized form.

Spencer[3] has shown that there are two critical periods in acute ferrous sulphate poisoning when death may occur. The first is within a few hours of taking the tablets and the second between 20 and 50 hours of ingestion. The cause of death in the first group has been ascribed to shock, the clinical picture of which was evident in the case we report and in the histological studies of the experimental animals. Smith,[4] basing his opinion on the work of Shorr *et al.*,[5] who showed that ferritin was a vasodepressant, considers that in iron poisoning excessive amounts of ferritin may be produced and released into the circulation, initiating and maintaining the vasomotor collapse characteristic of the early stages of the illness. However, the speed with which the shock was induced in the rabbits suggests a direct toxic action of the ferrous iron and leads us to put forward an alternative hypothesis: the hyperæmia and necrosis of the gastric mucosa which follow its exposure to large amounts of iron salts leads to a breakdown in the normal apoferritin-ferritin control mechanism. The plasma on becoming flooded with iron mobilizes both alpha and beta globulin to act as a protective ferric protein complex. Any iron left uncombined acts directly as a vasodepressant, thus precipitating the vascular collapse. Whether the vasomotor paralysis is central or peripheral we cannot say, although the absence of cerebral histological changes suggests the latter.

In the last analysis it may be that both excess circulating ferritin and uncombined iron cooperate in responsibility for this threat of death. In face of the evidence accumulating, there seems very little to support Spencer's suggestion that shock was an outcome of the large "wound area" in the upper alimentary tract following exposure to iron salts.

Alpha and beta globulin disappeared from the plasma of the rabbits and at the same time the concentration of iron in the reticulo-endothelial cells rose rapidly, suggesting that the globulins must enter these cells in order to deposit their iron and in doing so are destroyed. A certain amount of iron-bound protein was also lost by renal excretion. Replacement of protein is dependent on efficient synthesis; its production in the body is a function not only of the liver but also of certain extrahepatic tissues as well (Cheng[6]). It has been shown that the movement of large amounts of iron from the Kupffer to the parenchymal cells of the liver brings about cell necrosis by exposing the latter to iron in a free form. It would appear that the failure of hepatic function following necrosis plus the transient block of the reticulo-endothelial cells leads to a widespread depression of protein manufacture. This failure in synthesis was demonstrated in the rabbits by a concomitant rise in blood amino acid content; and in the patient the combination of protein loss and lack of production was so profound as to lead to an aproteinæmia which in turn led to tissue œdema. It is obvious that the changes in plasma protein will show variations in each case of iron poisoning, depending on its intensity. It should also be noted that in the livers of those animals sacrificed late in the experiment a normal histological picture was present when the globulin fraction of the plasma was still reduced and the blood amino acid concentration above normal. Nissim[7] considers that in iron poisoning functional damage is more serious than the histological appearances suggest.

The course of the patient's illness described in this report illustrated Spencer's second critical period. Forty-eight hours after the ingestion of iron he went into coma accompanied by convulsions. The changes occurred at the time of maximum hepatic damage and it would seem reasonable to consider that they probably arise from events following liver necrosis and blockage of the reticulo-endothelial system. The alteration in amino acid metabolism which followed could lead to glutamic acid deficiency, and, as glutamic acid is said to be necessary for the removal of the poisonous ammonium radicle in the central nervous system, its accumulation by the lack of an inactivator would result in the clinical picture described. It is of interest to note that ten hours after the start of glutamic acid therapy, the patient, although still very ill, had dramatically improved. It is our impression that sufficient liver function to maintain life will return with surprising rapidity, provided the patient can be tided over the period of hepatic coma.

A summary of the interpretation given to the facts gleaned clinically and experimentally is that, immediately after the ingestion and absorption of large amounts of iron, shock due to the presence of a circulating vasodepressant occurs;

subsequently there is protein loss by destruction and renal discharge, and at the same time protein synthesis is depressed by liver necrosis and the effects of reticulo-endothelial block. The destruction of protein and loss of synthesis lead to a hypoproteinæmia of varying degree accompanied by an alteration in amino acid metabolism, the latter manifested as a hepatic coma.

As regards treatment, the observations made suggest the necessity of early intravenous plasma infusion, probably of double or triple strength: firstly to combat shock, secondly to supply globulin for the absorption of "free" ferrous ions, thirdly to prevent osmotic imbalance which may be occasioned by protein loss. It is assumed that the preparation of dried plasma does not lead to the partial denaturation of protein which might invalidate its use for the second requirement. Whether vasomotor tone could be restored by the addition of noradrenaline (norepinephrine) to the infusion is worthy of consideration.

On theoretical grounds there seems indication for alternating the plasma infusion with one of casein hydrolysate. It is known that in Kinnier-Wilson disease copper chelates with amino acids for the purpose of elimination,[8] and it is possible that a similar mechanism took place between the hydrolysate given to the patient and the circulating iron, which may partly explain the unusually high urinary iron output seen on the fifth day of illness.

Although there is no proof that glutamic acid was responsible for the dramatic betterment that occurred after its exhibition following the diagnosis of hepatic coma, there is a good deal of evidence to suggest that its use was life-saving; consequently we feel it should be administered to any patient suffering from iron poisoning where hepatic coma has supervened.

There are two points in the general management of these cases that require mention. It has been shown that a transient, but profound, alteration takes place in the lung alveolar walls and leads to an alveolar exudate. The possibility of this becoming secondarily infected, leading to a bronchopneumonia, is apparent and at some period in the illness an antibiotic cover will be required. During the period of coma, aspiration of vomit is a Damoclean threat. The adoption of a tilted head-down bed, the provision of a sucker ready for instant use and a well-briefed nursing staff are necessities if a needless loss of life is to be averted.

In an analysis of accidental poisoning in childhood Craig and Fraser[9] write: "The only poison which is both common and very dangerous in Britain at present is ferrous sulphate." It would seem high time that some attempt was made at further preventive measures which might minimize to some extent the grievous harm that an accidental overdose may bring about. Whether it is possible to incorporate a small dose of an emetic, such as ipecacuanha, in each tablet—as is now being done in the barbiturates—is surely worth consideration.

SUMMARY

A case of acute iron poisoning which led to severe alterations in both plasma proteins and liver function is described. Experimentally it has been shown that a transient saturation of the reticulo-endothelial system with iron, necrosis of the liver, hypoglobulinæmia and raised blood amino-acid concentration follow acute ferrous sulphate poisoning in rabbits. Based on these findings, the mechanism of and therapeutic approach to acute iron poisoning are suggested.

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Ferrous Sulphate Poisoning.—N. F. ELLIOTT BURROWS, B.M. (for NORMAN HILL, M.D.)

T. W., aged 14 months.

Admitted to Belgrave Children's Hospital at 1.30 p.m. 7.11.50, with a history of having swallowed many $FeSO_4$ tablets one hour previously. Ten minutes later he had the first of a series of vomits, which produced no tablets, but only green-tinged fluid.

FIG. 1.—Ferrous sulphate tablets in stomach.

An immediate X-ray revealed what appeared to be 16 tablets in the stomach, and 3 or 4 others which had left it (Fig. 1).

The child was induced to vomit again by mechanical means, but no tablets were forthcoming. Vigorous washing out of the stomach was then undertaken with a total of 3-4 pints of sterile water. A brown liquid was obtained which gave a positive guaiacum test.

The child's condition gradually deteriorated, and by 3 p.m. he was extremely shocked pale, cold, clammy and comatose, with subnormal temperature, rapid thready pulse and rapid shallow respirations.

At 5 p.m. his condition had improved slightly and he passed a large black stool, which also gave a positive guaiacum reaction. By 7 p.m. he was not so well and becoming drowsy again. As the CO_2 combining power of his blood was only 42 vols.% he was given 0·75 gramme sod. bicarbonate hourly for five hours. By 10.30 p.m. he was markedly better, both pulse and respirations were almost normal, and although he passed 3 black stools during the next five days, he never gave any further anxiety.

His CO_2 combining power was 54·8 vols.% on the morning of 9.11.50.

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FERROUS SULFATE POISONING

WILLIAM M. CLARK Jr., M.D.
PORTLAND, ORE.
SEYMOUR S. JUROW, M.D.
NEW YORK
ROY L. WALFORD, M.D.
SAN DIEGO, CALIF.
AND
ROBERT O. WARTHEN, M.D.
WASHINGTON, D. C.

TOXIC effects from the ingestion of large doses of medicinal iron preparations have received increasing attention since 1947, when Forbes[1] reported two fatal cases. Since his report, 24 additional cases have appeared in the medical literature.* In all, 25 infants and 1 adult were involved. Ferrous sulfate was ingested in 25 cases and ferric chloride in 1 case. Thirteen cases were fatal, and in 13 there was recovery. There seems to have been no direct correlation between the amount of iron ingested and the final outcome, since the ingestion of as little as 3.0 gm. of ferrous sulfate has terminated fatally,[8] whereas the ingestion of 15.0 gm. has resulted in an uneventful recovery.[11]

Case 1 of the present study recovered after the ingestion of 2.4 gm. of ferrous sulfate. In Case 2, the ingestion of 10.2 to 14.2 gm. of ferrous sulfate proved fatal. The typical biphasic clinical course and the histopathologic findings are emphasized in the light of previous reports. The rationale of possible surgery in the future management of such cases is discussed.

REPORT OF CASES

CASE 1.—S. M., a 15-month-old white baby girl, was admitted to the Chanute Air Force Base Hospital on Dec. 12, 1952, with a history of having ingested eight 0.3-gm. enteric-coated ferrous sulfate tablets 17 hours previously. Two hours after ingestion of the tablets, she vomited partially digested food, but no blood or tablets were noted. The examination was negative at this time, except for listlessness. She was sent home on a milk diet, but the parents were advised to return if hematemesis or melena developed. That night the child slept fretfully and was irritable and feverish. Upon awakening in the morning, she passed a large pitch-black formed stool.

The physical examination upon admission revealed a temperature of 104 F., a mild and apparently subsiding right catarrhal otitis media, and marked irritability. The urinalysis was normal. The white blood cell count was 48,000, with 55% neutrophiles, 41% lymphocytes, 2% monocytes, and 2% eosinophiles. The hemoglobin was 11.0 gm. and the red blood cell count 3,500,000. The child was placed on a regimen of 300,000 units of aqueous penicillin procaine daily and given 15 cc. of liquid petrolatum (mineral oil) per os. A tap-water enema returned a small amount of formed grayish-colored stool. Her temperature returned to normal

From the Department of Pediatrics and the Laboratory Service, Chanute Air Force Base Hospital, Rantoul, Ill.

* References 2-14.

...after 24 hours, except for slight irritability on the subsequent days of hospitalization, was well developed, well nourished, and well hydrated. She was irritable, had normal stools, and did not vomit. The otitis media gradually cleared. On the second hospital day, the white blood cell count was 54,900, with 87% neutrophiles. On the third day, it was 5,650, with a normal differential count. She was discharged the following day with a normal blood cell count and has since remained well.

CASE 2.—A. L., a 20-month-old white girl, was admitted to the Chanute Air Force Base Hospital on March 17, 1953. She had been found playing with an empty pillbox four hours previously, and it was assumed that she had ingested from 34 to 44 0.3-gm. enteric-coated ferrous sulfate tablets. One hour after the ingestion, she vomited a yellow-green fluid with some partially digested food. No tablets were noted in the vomitus. Three hours after the ingestion, the vomiting had become almost continuous and was definitely bloody. Diarrhea supervened. The stools were greenish-black and liquid but contained no gross blood. Marked pallor developed, and the skin became cold and clammy. The child was brought to the hospital four hours after ingestion of the tablets.

Physical examination upon admission revealed an acutely ill infant who was vomiting blood-tinged fluid and had a bloody diarrhea. She was pale and mildly cyanotic and responded only to the strongest of stimuli. Her temperature was 101 F., blood pressure 70/0, and pulse rate 160. The abdomen was mildly distended. The remainder of the physical examination was negative. The white blood cell count was 12,100, the red cell count 4,100,000, the hemoglobin 12.0 gm., and the carbon dioxide combining power 27.5 vol. %.

She was placed in oxygen and given 150 cc. of 5% dextrose in isotonic saline, 250 cc. of 5% dextrose in water, 250 cc. of whole blood, and 250 cc. of 1/6 M sodium lactate intravenously; 300,000 units of aqueous penicillin procaine and 5 mg. of vitamin K were given intramuscularly. During and after the intravenous fluid therapy, the child became alert, responded to her parents, and asked for water (which was denied). The vomiting subsided, and there was a decrease in the bloody diarrhea. Her color improved, and the blood pressure rose to 120/70. Her temperature was 103 F. For the next six hours the child had less diarrhea, no vomiting, and a normal blood pressure. The red blood cell count was now 5,130,000, the hemoglobin, 13.5 gm., and the white cell count, 24,900, with 52% neutrophiles, 47% lymphocytes, and 1% eosinophiles.

About nine hours after admission, the child began to vomit again and developed an almost continuous bloody diarrhea. The vomitus contained clotted and liquid blood. At this time she was fed several strips of absorbable gelatin sponge U. S. P. (Gelfoam) moistened in isotonic saline. No change in the vomiting was seen. Another 250 cc. of whole blood was given intravenously. Although severe vomiting and diarrhea continued, the red blood cell count and the hemoglobin remained at 4,880,000 and 13.5 gm., respectively. Gradually, the blood pressure dropped until it was unobtainable and the pulse rose to 200. The child became cyanotic while in oxygen and deeply comatose and had repeated convulsions. Cheyne-Stokes respiration developed about 12 hours after admission, and she died 16 hours and 40 minutes after admission, or 20 hours and 40 minutes after ingestion of the tablets.

At postmortem examination, performed five hours after death, the body weighed 11,000 gm. and measured 83 cm. The pertinent gross and microscopic findings were as follows: A sharply localized segment of infarcted ileum was immediately evident upon opening the abdomen. The peritoneal fluid was scant. The blood in the larger vessels was fluid and without coagula.

The heart weighed 75 gm. The right auricle and ventricle were markedly dilated, and the pulmonary conus was moderately dilated. The right lung weighed 145 gm., the left lung 130 gm. Both lungs were boggy to palpation. The smooth mottled dusky-red surfaces revealed multiple 0.4-cm. areas of subpleural hemorrhage. On section, the dark red surfaces oozed considerable amounts of frothy reddish-white thin fluid. The mucosa of the left main-stem bronchus appeared dull and edematous, with small masses of brown semisolid material stuck to it. The liver weighed 440 gm. About the entrance of the portal vein and extending thence to involve roughly 30% of the liver the cut surface assumed a mottled brownish-grey color, appearing partially necrotic. In other areas the liver was grossly normal.

The esophagus was normal. The stomach contained about 10 cc. of a reddish-gray granular fluid material; however, the mucosa was well preserved. The duodenum and jejunum appeared normal. The entire small bowel measured 200 cm. in length. Eighty centimeters from its proximal

[illegible] small areas of necrosis and enlarged [illegible] Peyer's patches first appeared. This condition grew more pronounced for the next 20 cm., at which point the entire bowel wall abruptly assumed a purplish infarcted appearance. The strip of infarcted ileum was 15 cm. in length. The lumen was distended by reddish-purple granular necrotic semifluid material. The mucosa was entirely sloughed. Large ulcerated Peyer's patches were prominent (Fig. 1). The serosa

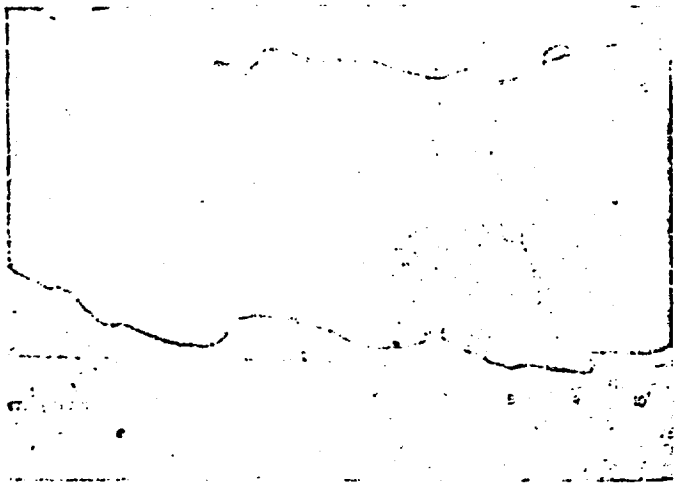

Fig. 1.—A segment of infarcted ileum, showing sloughed mucosa and two hyperplastic Peyer's patches with central ulceration.

Fig. 2.—A section of ileum from Figure 1, showing complete mucosal erosion, hyperplasia, and ulceration of Peyer's patch. The dark discoloration of the intestinal villi (iron pigment) is evident; × 440.

was dull and purple. The bowel mesentery at this point revealed dilated thrombosed venous channels. Eight to 10 large (up to 1.5 cm.) firm hemorrhagic lymph nodes were found adjacent to the region of infarcted ileum. The remainder of the small bowel showed similar but steadily diminishing [illegible] changes and was viable. The large bowel was normal.

Microscopic examination of the most severely involved portion of ileum revealed an edematous infarcted bowel wall [illegible] extending [illegible] the submucosa. The epithelium of the

villi and apical lamina was completely sloughed. The necrotic villi showed a marked brown granular discoloration, especially at their tips (Figs. 2 and 3). The submucosal vessels and the vessels of the lamina propria were dilated and contained masses of dark brown granular pigment. The pigment was most prominently distributed at the vessel periphery (Fig. 3). The Turnbull blue stain for ferrous iron was positive for this pigment and for that involving the

Fig. 3.—The ileum, showing thrombosed venous channels, with granular pigment material distributed at the vessel periphery. The pigment is iron-positive × 100.

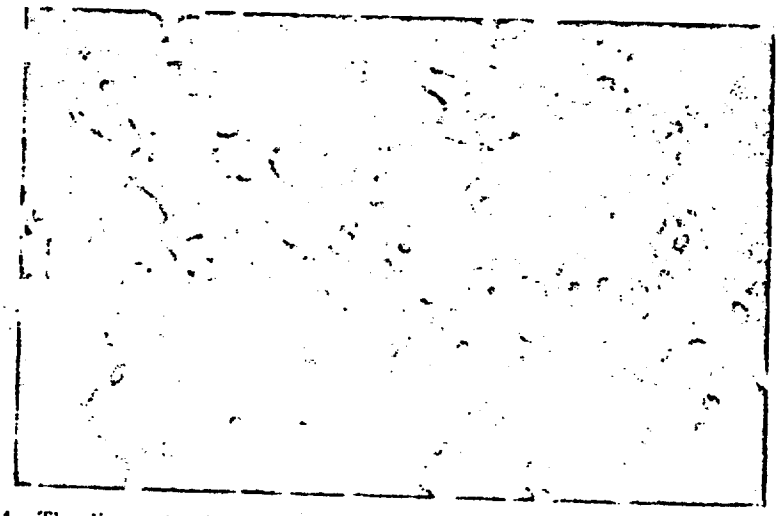

Fig. 4.—The liver, showing early necrotic changes manifested by cloudy swelling and dissolution of hepatic nuclei; × 440.

villi. There was profound hyperplasia, edema, and architectural distortion of the focal lymphoid follicles in the submucosa, with ulceration of overlying mucosa (Fig. 2).

In areas less severely involved, the edematous ileum showed sloughing of the epithelium over the villus tips; however, the epithelium at the bottom of the glands was preserved. Hyperplasia of Peyer's patches was also noted, but with less edema and no architectural distortion. The esophagus, stomach, and large bowel were normal.

The lymph nodes draining the infarcted segment were hyperplastic and hemorrhagic, with large distinct germinal centers. The wide, edematous, bloody lymphatic channels were jammed with neutrophiles, lymphocytes, and large mononuclear cells. No iron pigment could be demonstrated.

In the lungs, an extensive intra-alveolar hemorrhagic extravasation was noted. The alveolar walls were profoundly hyperemic. The bronchial epithelium was focally eroded and the small vessels greatly dilated. The peribronchial lymph nodes were hyperplastic and focally engorged with blood, round cells, and some neutrophiles. The hepatic sinusoids were engorged with blood. In areas, the parenchymal cells showed marked cloudy swelling, with focal dissolution of nuclei. The nuclear changes were characterized by marked peripheral clumping of chromatin, proceeding to disappearance of the nuclear membrane and scattering of chromatin material within the cell. The Turnbull blue reaction was negative in both lung and liver.

Permission to examine the brain was not granted. Other organs, including the kidneys, were normal grossly and microscopically.

A chemical analysis of the liver revealed 0.2 mg. of inorganic iron per gram. This is actually a low value. The gastric contents contained 1.2 mg. of inorganic iron per milliliter and the intestinal contents 1.1 mg. of inorganic iron per milliliter.

COMMENT

There is a fairly characteristic set of symptoms of iron poisoning. The clinical picture of Case 2 is similar to many of the reported cases. Within an hour of ingestion of the tablets, vomiting develops, which in two to four hours usually becomes bloody. The child becomes pale, irritable, and often comatose; the blood pressure falls, the pulse becomes rapid, and the child appears in profound shock. Diarrhea may or may not be present at this stage. Half of the reported fatalities were during this stage. Following this period of shock, there is often a rapid improvement in the clinical picture, during which the patient regains consciousness and is out of shock, and the vomiting and diarrhea decrease. However, in many cases there will occur a sudden relapse from 13 to 40 hours after ingestion, in which the child again goes into profound shock, with coma, severe bloody vomiting and diarrhea, and, frequently, convulsions.

The necropsy findings are also fairly characteristic. In all but one of the autopsied cases there was marked dilatation of the right heart, with pulmonary congestion and hemorrhage. Cloudy swelling or early necrosis of the liver were frequently observed. In general, a hemorrhagic necrotizing gastritis followed the ingestion of plain coated tablets, whereas a similar localized enteritis resulted from the ingestion of enteric-coated tablets, due to their liberation in the lower gastrointestinal tract. Three of the fatal cases involved enteric-coated tablets; one of these was associated with necrosis of the gastric mucosa, and the other two with necrosis of the small intestine. Plain tablets were ingested in the remaining 10 fatal cases, and all of these showed necrosis or marked congestion of the gastric mucosa. Two of these also showed necrosis of the small intestine, one having ingested approximately 20 gm. of ferrous sulfate and the other 240 gm. In our second case, it would appear that the enteric-coated tablets proceeded down the intestinal tract relatively intact until the enteric coating was finally dissolved at the isolated segment of necrotic ileum.

Case 1 is interesting because of the high white blood cell count, of 54,900, which persisted for about 60 hours. In Case 2, the white cell count reached 24,900. Marked

leucocytosis has also been reported in some of the other cases: 37,800, in the case of Swift and co-workers,[15] 21,750, in one of Duffy and Diehl's cases,[12] 79,000, in Lindquist's case,[7] and 55,750, in Foucar's case.[8] The significance of these elevations is unknown.

The treatment of iron poisoning is largely symptomatic. Immediate efforts should be made to make the child vomit in order to rid the stomach of any undissolved tablets or fragments of tablets. In addition, copious and prolonged gastric lavage should be done with sodium bicarbonate solution, leaving some in the stomach in an effort to convert the ferrous sulfate to insoluble ferrous carbonate. Shock should be combated with blood, plasma, and oxygen as indicated, and one should be alert for the possibility of a delayed exacerbation after initial improvement. Roxburgh[5] used dimercaprol (BAL) in the treatment of a 16-month-old patient who recovered from iron poisoning, but he made no claim for benefit from it. Animal experimentation indicates that the toxic effects of ferrous sulfate are aggravated by dimercaprol.[6] Spencer[8] has suggested use of the following prescription in cases of iron poisoning:

Thiamine hydrochloride	10 mg.
Nicotinamide	30 mg.
Riboflavin	10 mg.
Tocopherol	15 mg.
Methionine	500 mg.

Multiply the above amounts by the patient's age in years and give in three divided doses daily.

It is suggested that the iron, acting as a heavy metal, may combine with —SH groupings and thus interfere with oxidation. The tocopherol is given in an effort to reduce the oxidative requirements of the cells and the methionine as a source of —SH groupings and as protection for the liver.

It is well known to radiologists that enteric-coated iron tablets are radiopaque. If an x-ray of the abdomen reveals iron tablets grouped together in the intestine, a localized patch of gangrene of the intestine such as was found in our second case is to be expected. If so, a laparotomy should be considered after recovery from the initial shock stage to remove the tablets and to resect the necrotic segment of bowel.

SUMMARY

Two cases of iron poisoning are presented. The clinical features and pathology of the condition are reviewed and the available forms of treatment discussed. The use of surgery in selected cases is suggested.

The photographs of Case 2 were taken by Technical Sergeant Roland J. Englehardt, University of Oregon Medical School, Portland, Ore. (Dr. Clark).

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Ferrous sulfate poisoning

A review, case summaries, and therapeutic regimen

The incidence, pathology, and symptoms of acute severe iron poisoning are reviewed. Four cases are presented: the first terminated in death by acute hepatic failure; the second case with severe first and second phase symptoms was treated successfully with peritoneal dialysis and calcium disodium EDTA; and in the third and fourth cases recovery occurred after treatment by chelation and supportive means. The clinical phases of acute iron poisoning are reviewed, and a logical plan for management is formulated.

Thomas J. Covey, M.D.*

VALPARAISO, IND.

FERROUS sulfate poisoning was first reported in American medical literature in 1850.[1] One half of the 42 recorded poisonings in children occurring in the period from 1947 to 1956 were fatal. The smallest dose of ferrous sulfate resulting in death in this series was 3 Gm. while as much as 15 Gm. were ingested with recovery. In animals the fatal dose is calculated to be 150 to 200 mg. per kilogram.[2] It is probably safe to assume that as little as 1 Gm. can be fatal to a child.[3]

Iron is an important cause of accidental poisoning in children in England with the frequency being comparable to aspirin poisoning in the United States.[4] From 1930 to 1953, fifty-three deaths were recorded in Great Britain. The span from 1950 to 1953 accounted for thirty-two of these fatalities.

From the Department of Pediatrics and the Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Ill.

Address, 1 Sheffield Drive, Valparaiso, Ind.

In addition to establishing the minimal probable fatal dose of ferrous sulfate in children, the Nineteenth Ross Pediatric Research Conference further correlated symptoms and sites of pathology and suggested a specific treatment not previously used. Three critical phases of severe iron poisoning were described. The early acute phase with signs of vomiting of fresh or altered blood, diarrhea and melena accompanied by shock, coma and acidosis occurs within one-half to one hour of ingestion. Recurrent shock constitutes the second phase and occurs 20 to 48 hours after ingestion. Aldrich classified these phases as initial cardiovascular collapse with death in 6 hours or less if the patient cannot be supported, a period of deceptive improvement for 10 to 11 hours and a recurrent phase of severe usually irreversible shock within 20 hours of ingestion. Edathamil calcium disodium EDTA was recommended as a possible chelating agent.[3]

[illegible] vomiting, and constipation with pyloric obstruction is also to be anticipated. Gandhi and Robarts[6] analyzed the phase of gastric obstruction. In 11 instances, the average interval of symptoms of obstruction from time of ingestion of ferrous sulfate was 4 weeks with a range of 13 to 40 days. Five cases had pure pyloric stenosis resulting from the direct corrosive action of ferrous sulfate on the gastric mucosa. The remaining 6 children had gastric stricture and in 2 instances had pyloric stenosis as well. One child, in addition to a stricture, had a gastric ulcer penetrating into the liver.

At the Cook County Children's Hospital in Chicago, Ill., 1,427 children who ingested potentially poisonous substances were seen from Jan. 1, 1962 to July 1, 1963, and the material involved in 20 instances was iron.[7] One fatal and three severe cases of iron poisoning from this group will be presented and the therapy outlined.

CASE REPORTS

Case 1. A. W., a 16-month-old Negro male was admitted to Cook County Children's Hospital at 2:15 A.M. on Oct. 15, 1962, five days after he had taken 20 of his mother's ferrous sulfate tablets, size unknown. In the succeeding days he developed anorexia, fever, and lethargy; jaundice was noted on the day before admission.

Physical examination revealed an acutely ill child with temperature of 103.8° F., pulse rate of 110, respiratory rate of 40, and weight 10 kilograms. Sclerae and skin were markedly icteric, and there was fetor hepaticus. The liver was firm and enlarged to 6 cm. below the right costal margin, and the spleen was palpable by 2 cm. In spite of supportive treatment with intravenous fluids, tetracycline and oxygen, he died 12 hours later.

On admission the complete blood count was: hemoglobin 4.2 Gm., red blood cells 1,500,000, white blood cells 102,000 (corrected 61,450) with 29 neutrophils, 21 band forms, 23 lymphocytes, 8 monocytes, 3 myelocytes, 20 metamyelocytes, and 65 nucleated red blood cells. Other laboratory reports were 2-plus urobilinogen in the urine, serum bilirubin 29 mg. per 100 ml. with 3.2 and 3.6 mg. per 100 ml. direct, cephalin flocculation 4-plus, thymol turbidity 4 units, [illegible] and nonprotein nitrogen 67 mg. per

Significant autopsy findings were [illegible] tration and massive necrosis of the [illegible] right lobe of the liver with heme[illegible] posited in the fibrous septa. There w[illegible] rhages and corrosive changes in t[illegible] and first part of the duodenum, leuk[illegible] tion in the bone marrow, and aspir[illegible] chopneumonia.[8]

Case 2. M. P., a 23-month-old N[illegible] was admitted to Cook County Chil[illegible] pital on Feb. 19, 1963, at 4:45 P.M. [illegible] ingested a minimum of 25 to a maxi[illegible] ferrous sulfate tablets, size unknown, [illegible] noon, and within one or two hours [illegible] vomiting black material streaked with [illegible] had passed several black liquid stool

Physical examination revealed an [illegible] lethargic child with blood pressure 95 [illegible] pulse 100, temperature 99° F., and [illegible] kilograms. Fifteen minutes later she [illegible] tose and unresponsive to stimuli. Gas[illegible] was immediately begun by using 3.[illegible] sodium bicarbonate in tap water and [illegible] until the black bloody return became [illegible] other 3.75 Gm. of sodium bicarbona[illegible] c.c. of milk of magnesia were left in [illegible] ach, and intravenous fluids were st[illegible] serum iron level at this time was late[illegible] as 1,166 μg per 100 ml. and the hema[illegible] 43 per cent. A peritoneal catheter w[illegible] two and a half hours after admission, [illegible] toneal dialysis was begun with the use[illegible] tion containing approximately 370 m[illegible] liter and electrolyte composition [illegible] plasma except for the absence of [illegible] Dialysis solution in 400 c.c. amount[illegible] tilled and withdrawn every 45 to 6[illegible] Attempts to use a larger volume seem[illegible] nificantly impair ventilation. Tetracy[illegible] added to the intravenous fluids becau[illegible] possibility of aspiration during gastr[illegible] Bloody emesis of about 200 c.c. oc[illegible] hours after admission, and 200 c.c. [illegible] blood was given with stabilization of [illegible] Fifteen hours after dialysis was begun[illegible] gan leaking from the site of the cath[illegible] tion and limited instillation of dialys[illegible] 300 c.c. increments. This occurred [illegible] the usual purse-string procedure as a [illegible] catheter was not avilable and an adul[illegible] had to be used.

The patient developed profound sl[illegible]

A late phase of gastric scarring and contracture with pyloric obstruction is also to be anticipated. Gandhi and Robarts[6] analyzed the phase of gastric obstruction. In 11 instances, the average interval of symptoms of obstruction from time of ingestion of ferrous sulfate was 4 weeks with a range of 13 to 40 days. Five cases had pure pyloric stenosis resulting from the direct corrosive action of ferrous sulfate on the gastric mucosa. The remaining 6 children had gastric stricture and in 2 instances had pyloric stenosis as well. One child, in addition to a stricture, had a gastric ulcer penetrating into the liver.

At the Cook County Children's Hospital in Chicago, Ill., 1,427 children who ingested potentially poisonous substances were seen from Jan. 1, 1962 to July 1, 1963, and the material involved in 20 instances was iron.[7] One fatal and three severe cases of iron poisoning from this group will be presented and the therapy outlined.

CASE REPORTS

Case 1. A. W., a 16-month-old Negro male was admitted to Cook County Children's Hospital at 2:15 A.M. on Oct. 15, 1962, five days after he had taken 20 of his mother's ferrous sulfate tablets, size unknown. In the succeeding days he developed anorexia, fever, and lethargy; jaundice was noted on the day before admission.

Physical examination revealed an acutely ill child with temperature of 103.8° F., pulse rate of 140, respiratory rate of 40, and weight 10 kilograms. Sclerae and skin were markedly icteric, and there was fetor hepaticus. The liver was firm and enlarged to 6 cm. below the right costal margin, and the spleen was palpable by 2 cm. In spite of supportive treatment with intravenous fluids, tetracycline and oxygen, he died 12 hours later.

On admission the complete blood count was: hemoglobin 4.2 Gm., red blood cells 1,500,000, white blood cells 102,000 (corrected 61,150) with 25 neutrophils, 21 band forms, 23 lymphocytes, 8 monocytes, 3 myelocytes, 20 metamyelocytes, and 65 nucleated red blood cells. Other laboratory reports were 2-plus urobilinogen in the urine, serum bilirubin 29 mg. per 100 ml. indirect and 3.6 mg. per 100 ml. direct, cephalin flocculation 4-plus, thymol turbidity 4 units, gamma globulin turbidity over 3.2 Gm. per cent, and nonprotein nitrogen 67 mg. per cent.

Significant autopsy findings were fatty infiltration and massive necrosis of the caudate and right lobe of the liver with hemosiderin deposited in the fibrous septa. There were hemorrhages and corrosive changes in the stomach and first part of the duodenum, leukemoid reaction in the bone marrow, and aspiration bronchopneumonia.[8]

Case 2. M. P., a 23-month-old Negro female was admitted to Cook County Children's Hospital on Feb. 19, 1963, at 4:45 P.M. She had ingested a minimum of 25 to a maximum of 40 ferrous sulfate tablets, size unknown, that afternoon, and within one or two hours had begun vomiting black material streaked with blood and had passed several black liquid stools.

Physical examination revealed an acutely ill, lethargic child with blood pressure 95/50, apical pulse 100, temperature 99° F., and weight 10 kilograms. Fifteen minutes later she was comatose and unresponsive to stimuli. Gastric lavage was immediately begun by using 3.75 Gm. of sodium bicarbonate in tap water and continued until the black bloody return became clear. Another 3.75 Gm. of sodium bicarbonate and 45 c.c. of milk of magnesia were left in the stomach, and intravenous fluids were started. The serum iron level at this time was later reported as 1,166 μg per 100 ml. and the hematocrit was 43 per cent. A peritoneal catheter was inserted two and a half hours after admission, and peritoneal dialysis was begun with the use of a solution containing approximately 370 mOsm. per liter and electrolyte composition similar to plasma except for the absence of potassium. Dialysis solution in 400 c.c. amounts was instilled and withdrawn every 45 to 60 minutes. Attempts to use a larger volume seemed to significantly impair ventilation. Tetracycline was added to the intravenous fluids because of the possibility of aspiration during gastric lavage. Bloody emesis of about 200 c.c. occurred 11 hours after admission, and 200 c.c. of whole blood was given with stabilization of vital signs. Fifteen hours after dialysis was begun fluid began leaking from the site of the catheter insertion and limited instillation of dialysis fluid to 300 c.c. increments. This occurred even with the usual pursestring procedure as a pediatric catheter was not available and an adult catheter had to be used.

The patient developed profound shock with

hematocrit of 67 per cent 30 hours after admission, but she improved following administration of 200 ml. of plasma. Calcium disodium EDTA, 350 mg., intramuscularly, every 12 hours for 10 doses was begun 2 hours later. The next day the patient developed signs of consolidation of the right middle lobe and the peritoneal fluid was cloudy. A Gram stain of the peritoneal fluid was interpreted as gram-positive diplococci; the intravenous antibiotic was changed to aqueous penicillin G, 5 million units per 24 hour periods, and the dialysis was discontinued. Ileus with abdominal distention which followed was relieved with continuous Levin suction and fluid and electrolyte replacement. The potassium at that time was 5.7 mEq. per 100 ml. so hypokalemia was not thought to be the etiology of the ileus. When gram-negative rods, later identified as *Escherichia coli* sensitive to chloramphenicol and colistin, were discovered in the dialysance culture, intravenous antibiotic therapy was changed to chloramphenicol.

By February 27 this patient had improved enough to take fluids orally. The antibiotic was continued for a total of 8 days until March 4. However, after chloramphenicol was stopped, fever, abdominal pain, and distention returned, and colistin was given, 25 mg. intramuscularly every 12 hours for 6 days. The patient (M. P.) was markedly improved by March 9 and continued to remain well. Urine culture grew *Staphylococcus aureus* and enterococci, sensitive to nitrofurantoin and with colony count over 10^5 on March 18, 1963, and nitrofurantoin was given orally on March 21 for 10 days.

incidentally: oxygen from the time of admission to February 27; antacid therapy with aluminum hydroxide gel from February 20 to March 18; daily intravenous and then oral vitamins, and supportive nursing care. An upper gastrointestinal series was normal one month after admission, and the patient was discharged in good condition, apparently recovered. Laboratory data are summarized in Table I, and the most interesting data concerning dialysis and values of iron in serum, urine, and dialysance are to be found in Table II.

Case 3. A. L., a 21-month-old Negro male was admitted to the Cook County Children's Hospital on May 21, 1963, at 11:00 P.M., 4 hours after ingesting an unknown number of iron and calcium tablets, probably less than 15, prescribed for his mother. The amount of iron contained was not known. Two and one half hours after ingestion he regurgitated about 10 tablets and then vomited twice more.

Physical examination showed a child of 12 kilograms with temperature of 97° F., blood pressure of 100/60, pulse of 120, and shallow respirations of 30 per minute. He was semistuporous but responded to pain. His pupils were miotic. The abdomen was soft with hyperactive bowel sounds. After digital rectal examination there was expulsion of a watery black stool. Deep tendon reflexes were not elicited. The patient was lavaged with sodium bicarbonate and given an enema of normal saline and sodium bicarbonate. An admission serum iron concentration was 463 gamma per 100 ml., the hematocrit was 33 per cent, carbon dioxide [illegible]

Table I. Summary of laboratory data. Patient M. P.

Date	*BUN (mg. %)*	*Na (mEq.)*	*Cl (mEq.)*	*K (mEq.)*	*CO_2 (mEq.)*	*Prot. A/G (Gm. %)*	*Icteric index*
2/20	10	130	97	5.2	17.3	4.2/2.4	6
2/21		135	99	8.1		5.2/2.6	10
2/22	22	131	95	5.7		4.4/2.8	
2/23	52	136	91	5.7			
2/25	13	139	94	3.2	32.7	3.1/2.3	11
2/26							
2/27	6	135	85	5.3	30.0		15
2/28	7	128	90	4.3			14
3/1	9	131	89	3.7			8
3/4	11	128	90	4.3			
3/5							6
3/7	12						6
3/11	16						
3/22						4.0/3.9	6

mEq [illegible], and nonprotein nitrogen 20 mg. per 100 ml. Intravenous fluids and oral aluminum hydroxide gel were initiated. Calcium disodium EDTA was given, 450 mg., intramuscularly, every 12 hours, beginning 2 hours after admission. On May 22 at 7:15 A.M. he was awake and crying. Vital signs had remained stable and he voided for the first time since admission. The urine contained 160 gamma iron per 100 ml., and a serum iron obtained shortly afterward was 181 gamma per 100 ml. The intravenous fluids were discontinued later that evening, but calcium disodium EDTA was continued for a total of 5 days along with oral vitamins and antacid treatment. A flat film of the abdomen showed contrast material remaining in the stomach and rectosigmoid areas even after two 500 c.c. enemas of normal saline on May 21. He was discharged on May 26 in good condition.

Serum iron on May 23, thirty-eight hours after ingestion, was 65 gamma per 100 ml. and a urine at that same time contained 800 gamma iron per 100 ml. The iron binding capacity was 217 gamma per 100 ml. and total iron binding capacity 282 gamma per 100 ml. with 23 per cent saturation. The urinalysis was normal on May 23 except for 2-plus coproporphyrin III. Four serial liver profiles, including serum proteins, icterus index, cephalin flocculation, thymol turbidity, gamma globulin turbidity, and alkaline phosphatase, were normal. Hematologic studies showed: Hemoglobin 11.2 Gm., red blood cells 4.05 million, white blood cells 15,100 with a shift to the left.

Case 4. A. H., a 17-month-old Negro male was admitted to the Cook County Children's Hospital on May 22 at 7:15 P.M., 1 hour after ingesting 90 Mol-iron tablets (195 mg. ferrous sulfate per tablet). He had vomited spontaneously at least 5 times after taking the pills, and in the admitting pavilion he was lavaged and an universal antidote was instilled into his stomach.

Admission examination revealed a child of 10 kilograms with apical pulse of 120 and respirations of 28. He was apparently in good health and showed no significant findings other than mild lethargy and the vomiting of clear material with dark flecks of blood. Maintenance intravenous fluids with 750 mg. of EDTA for 24 hours were started after obtaining an initial serum iron of 592 gamma per 100 ml. The child was placed in an oxygen tent and given 5 mg. of vitamin K_1 oxide, intramuscularly, and oral aluminum hydroxide gel. Four hours after ingestion the child was very lethargic and difficult to arouse, but the blood pressure and other vital signs were stable. No diarrhea had occurred so he was given a normal saline enema. Fifteen hours post ingestion he was still lethargic but otherwise well, and a flat film of the abdomen did not show radiopaque material. A repeat serum iron at that time was 400 gamma per 100 ml. and the first urine was voided which contained 200 gamma of iron per 100 ml. By 28 hours he was alert but irritable, voiding and apparently normal. Another 750 mg. dose of intravenous EDTA was placed in the maintenance fluids for the next 24 hour period. Following this, fluids were given orally and EDTA was continued, 375 mg. being given intramuscu-

Cephalin flocculation	*Thymol turbidity (Mac L. U.)*	*Gamma globulin turbidity (Gm. %)*	*Alkaline phosphatase (Bodansky unit)*	*SGOT/SGPT*	*WBC*	*Hb (Gm.)*
[illegible]-plus	3.0	1.10			21,400	15.8
[illegible]-plus	3.1	0.85				
[illegible]-plus	2.6	0.70	6.5			
[illegible]-plus	3.8	1.20	9.4		18,200	10.8
[illegible]-plus	5.0	0.90				
[illegible]-plus	4.1	0.70		30/9		9.7
[illegible]-plus	6.8	1.20			18,100	
O[illegible]	4.8	1.35	7.5	26/23		

Table II. Summary of dialysis data and values of iron* in serum, urine and dialysance

Date	Dialysis: In (c.c.)	Dialysis: Out (c.c.)	Dialysis: Test (c.c.)	Total Fen† gamma test	24 H. U. Vol. (c.c.)	Total Fec‡ gamma	% Fes§ gamma	% Fen test gamma	% Fec test gamma	Total Fen out gamma (calc.)
2/19	1,600	1,500	5,738‖	529.1			1,166	9.2		697.8
2/20	7,700	6,085	L ¶							
2/21	V 5,700	5,140	L 5,717**	818.9	470	380	168	14.3	80.9	777.9
2/22	V 2,800	2,500	L 1,900***	302.7	330	260	203	15.9	78.8	397.5
2/23	V									
2/24	V									
2/25	V						47			
Total	17,800	15,525	L 13,355	1,750.7						1,873.2

Inter-relationships of iron in serum, urine and dialysance: On 2/21/1963 and 2/22/1963 when simultaneous serum, urine, and dialysance iron levels were obtained, 1175.4 gamma Fe was removed in dialysance (calculated) and 640 gamma Fe was excreted in the urine. Fe was removed in dialysance, 697.8 gamma calculated, even before calcium disodium EDTA was given. This chelating agent increased the iron removed in dialysance by at least 55% even in the presence of a normal serum iron level.

*Fe determinations by laboratory of R. J. Dern, M.D., Hektoen Institute for Medical Research, Chicago, Ill.

**2/21 and part of 2/20

***discontinued at 5:00 P.M. (peritonitis)

†Fen = Total gamma or gamma% Fe in dialysance

‡Fec = Total gamma or gamma% Fe in urine

§Fes = Gamma% serum Fe

±V = Calcium disodium EDTA begun at noon

¶L = Unknown amount of leakage

‖2/19 and part of 2/20

larly every 12 hours for 6 doses. Aluminum hydroxide gel was continued at intervals of 4 hours and vitamins were given daily. A serum iron at 40 hours was 10 gamma per 100 ml. and at 64 hours was 9 gamma per 100 ml. with iron binding capacity of 364 gamma per 100 ml., total iron binding capacity of 373 gamma per 100 ml., and saturation of 2 per cent.

Other laboratory tests were: normal urinalysis on May 23; hemoglobin of 5.4 Gm. per 100 ml., red blood cells 3.44 millions, and white blood cells 8,600 with normal differential. There were 3-plus hypochromia, 1-plus microcytosis, and 2-plus anisocytosis noted. The hemoglobin rose to 6.1 Gm. on May 28 and 6.8 Gm. on May 31. Liver profiles, including those noted in Case 3, were borderline normal on 5 different occasions. Electrolytes and blood urea nitrogen were normal except for a carbon dioxide of 19.5 mEq. on May 23. His course was uneventful, and he was discharged on June 4 to return for readmission on June 23. At this time there were no symptoms, and the physical examination was normal. Hemoglobin was 8.5 Gm. per 100 ml., red blood cells 5.04 millions, white blood cells 12,400, and differential normal except for 7 per cent eosinophils. There was 3-plus hypochromia, 2-plus anisocytosis, and 1-plus microcytosis. Liver profile and upper gastrointestinal series were both normal.

DISCUSSION AND CONCLUSIONS

The treatment of iron poisoning by means of chelation with EDTA is well grounded. Theoretically, this chelating agent should form a soluble, relatively nonionizable, not too toxic compound in its combination with iron and thus be available for excretion. Experimentally, calcium has been shown to be less strongly bound to EDTA than iron.[9] In fact, this was shown to be true clinically by Wishinsky and his co-workers[10] who used calcium disodium EDTA to mobilize and remove iron in an adult with hemochromatosis. Their patient's excretion of iron was 1.2 mg. per day or almost 4 times normal. With daily intravenous administration of the chelating compound this baseline excretion of iron was increased threefold. The only untoward effect was a rapid fall in prothrombin activity. Bronson and Sisson[11] demonstrated in dogs severely intoxicated with iron that calcium disodium EDTA lowered serum iron concentration and prolonged survival time. One case of iron poisoning in a child was managed with this agent but the child died 60 hours after ingestion of the iron.[12]

Two children with severe iron poisoning recovered when Schafir[13] first successfully used the recommended one dose calcium disodium EDTA treatment of 80 mg. per kilogram, one half the dose being given intravenously and one half orally after removal of as much ferrous sulfate from the gastrointestinal tract as possible and administration of oral sodium bicarbonate to form an insoluble iron compound. The initial serum iron level in the first case was 6,080 gamma per 100 ml. and by the fourth day had decreased to 320 gamma per 100 ml. The only urine value obtained was 90 gamma iron per 100 ml. on the eleventh day when a simultaneously obtained serum iron was 256 gamma per 100 ml. Schafir's second case had a first day serum iron level of 373 gamma per 100 ml. which decreased to 200 on the second day when the urine iron was 217 gamma per 100 ml. Another child was successfully treated by Barrie and Wilson.[14] The initial iron level was 4,840 gamma per 100 ml. and 36 hours after admission the value had declined to 137. Very significantly, they demonstrated that when all recorded cases of iron poisoning that had serum iron levels reported were graphed, the levels followed the same pattern regardless of treatment. That is, a steep decline in serum iron occurred in 36 hours with the lowest levels present in the patients treated with EDTA. Data on urine iron concentration in cases of poisoning are limited to the previously mentioned instances in man. Foreman and coworkers[15] showed that excretion of radioactive iron in the rat was doubled by chelating agents.

From the aforementioned evidence it is logical to conclude that the use of calcium disodium EDTA is of great value in the treatment of acute iron poisoning. However, when this is used as the only means of removal of absorbed iron, the sole avenue of excretion is by way of the urine. Since the first two phases of shock may compromise blood flow to the kidney and reduce urine volume, it becomes evident that this mode of excretion will be impaired. Furthermore, since there is a sharp decline in serum iron within 36 hours regardless of treatment, it is quite likely that this represents diffusion into tissue. Thus, a single dose of 500 mg. of intravenous calcium disodium EDTA as recommended may not be enough to chelate all the excess iron present. There are theoretical objections to the oral use of this compound as the chelate is soluble and may be partially ionizable. Thus, an additional potentially toxic quantity of iron might be absorbed through the intact lower small bowel.

Other methods with or without the use of EDTA have been tried or suggested to remove absorbed iron after acute poisoning. Amerman, Brescia, and Aftahi[16] in 1958 used exchange transfusion in treating a child who recovered as did Weichsel[17] in 1962. Combined chelation, hemodialysis, and alkalinization as a treatment for iron poisoning was suggested by Felts, Barringer, and Meridith[18] on the basis of in vitro experiments. They found it possible to dialyze ferric ammonium citrate from saturated reservoirs and recoveries were 25 and 57 per cent of added iron. When calcium disodium EDTA was infused directly into the reservoirs, the yield was increased to 44 per cent and 69 per cent after 4 hours of dialysis. Under ideal conditions, in severe iron poisoning when urine flow is markedly reduced, intravenous calcium disodium EDTA and hemodialysis appear to be the treatment of choice in removing absorbed iron. However, this is not readily available except in large medical centers, and precious time in early critical hours after iron poisoning is consumed in setting up this procedure.

Peritoneal dialysis suggests itself as a simple, almost universally available technique for removal of iron. Data from Case 2 tabulated previously show that a significant amount of iron was removed by dialysis alone in the first 40 hours. After administration of intramuscular calcium disodium EDTA, the concentration of iron in dialysance was increased by 55 per cent even in the presence of a normal serum iron level. On February 21, when serum, dialysance, and urine iron levels were simultaneously obtained, the calculated total dialysance

...apy could be summarized in this manner:

1. Induce emesis with patient in prone position and neck flexed to prevent aspiration, and then initiate gastric lavage with sodium bicarbonate. Leave a solution of sodium bicarbonate and a saline cathartic in the stomach.

2. Intravenous fluids should be given as soon as possible and should contain intravenous calcium disodium EDTA, 50 to 75 mg. per kilogram per day divided into two doses to chelate all possible free absorbed iron. After the second shock phase is passed, this compound may be given intramuscularly for a total of 4 or 5 days. This agent should be administered even in the absence of symptoms if there is a well-documented history of ingestion of one or more grams of ferrous sulfate or the amount is unknown. Calcium disodium EDTA should be continued until serum iron or urinary iron values are known, if they can be obtained readily or until 48 hours have elapsed since the time of poisoning and second phase symptomatology has not occurred.

3. Blood, plasma, and vasopressors in addition to intravenous fluid replacement and supportive measures should be used for any sign of cardiovascular collapse.

4. Peritoneal or hemodialysis may be considered in addition to the aforementioned measures if severe first phase symptoms are present and urine flow is greatly reduced or absent.

5. Intravenous and oral vitamins may help to prevent liver damage, and oxygen may afford further protection during the first 48 hours after poisoning.

6. Laboratory determinations should include serum proteins when dialysis is used, routine electrolytes to guide in maintenance fluids and treatment of acidosis, liver function tests to detect signs of hepatic injury, and at least a serum iron drawn on admission.

7. Upper gastrointestinal series at 4 weeks can in the absence of gastric obstruction ... be strongly recommended to rule out peptic ulceration or damage to the stomach or duodenum. Laparotomy should be considered if persistent vomiting occurs any time after the second week post poisoning and an upper gastrointestinal series corroborates the diagnosis of obstruction. Early and continuous antacid and dietary therapy may help in prevention of this complication.

Cases 1 and 4 were from the service of Dr. Joseph Greengard, Chairman of the Department of Pediatrics, and Dr. Matthew Lewison. Case 2 was from the service of Dr. Rowine Hayes Brown, and Case 3 was from the service of Drs. Ira M. Rosenthal and Ronald B. Mack. These attending pediatricians kindly permitted publication of the aforementioned cases. Dr. Paul Szanto, Chairman of the Department of Pathology, kindly permitted publication of the autopsy findings of Case 1.

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J.A.M.A., Dec. 4, 1954

156 : 1326-28

FATAL CASE OF IRON INTOXICATION IN A CHILD

Charlotte D. Curtiss, M.D.
and
Alexander A. Kosinski, M.D., Johnson City, N. Y.

Acute iron intoxication in children has now been sufficiently well documented to have become a readily recognized clinical entity; it seems worth while to report this additional case because the number of recorded cases is still small and because this case presents the unusual features of severe hepatic and renal damage.

REPORT OF A CASE

The patient was a 21-month-old white girl who was admitted to the Charles S. Wilson Memorial Hospital on June 7, 1953, with a history of having ingested a large (but unknown) number of iron-containing capsules about eight hours previously. (The composition of these capsules is given by the manufacturer as dried ferrous sulfate 0.162 gm., liver concentrate N.F., 0.17 gm., and dried yeast.) Before admission the child had been vomiting and having diarrhea and had gradually lapsed into unconsciousness. On arrival at the hospital, she appeared lethargic and dehydrated. She was in shock; no blood pressure was obtainable, the pulse was rapid, and the skin was cool. Her pupils were dilated and reacted sluggishly to light. Noisy, irregular respirations and coarse bronchial rales were heard. Plasma, blood, and other fluids were given intravenously. The patient appeared to rally but continued to have intermittent hematemesis and bloody diarrhea. Approximately 48 hours after she had ingested the pills, the child died suddenly in acute pulmonary edema.

Autopsy disclosed diffuse and severe congestion of all the viscera. Petechial hemorrhages were noted in the pericardium, pleurae, thymus, and adventitia of the aorta. The right and left lungs weighed 195 gm. and 160 gm., respectively. They were bulky, congested, and readily oozed blood and foam. Although the gastrointestinal tract was patent throughout, superficial hemorrhages were present in the mucosa of the stomach, lower ileum, and rectosigmoid colon. In addition, two superficial dark gray ulcerations, each about 2 mm. in diameter, were found in the fundus of the stomach. The liver weighed 480 gm. Its inferior aspect showed an orange-yellow speckling, which on section was seen to extend deep into the parenchyma. The organ was rather soft and intensely congested. The kidneys were unusually heavy,

From the Charles S. Wilson Memorial Hospital Pathology Laboratory.
Dr. Raeburn Wharton gave permission to report this case.

weighing 80 and 60 gm. The cortices, bulging and yellowish-tan, were sharply demarcated from the congested medullae. Blood studies performed on specimens obtained at autopsy (two hours post mortem) revealed a nonprotein nitrogen level of 121 mg. per 100 cc. and a serum chloride level of 540 mg. per 100 cc. The Wassermann reaction was negative.

Microscopic examination generally confirmed the gross findings. The lung parenchyma and pleurae were congested, focally hemorrhagic, and edematous. No pneumonic process was observed; however, small lymphocytic infiltrations around the bronchi were considered evidence for an unrelated chronic bronchitis. The stomach and small intestine showed mucosal lesions that were largely limited to the superficial portions bordering the lumen. Occasionally the necrosis extended more deeply into the mucosa, forming small focal ulcerations covered by a purulent exudate. Diffuse congestion, edema, and tiny hemorrhages were found throughout the sections. Considerable accumulations of dark brown granular pigment were noted within the mucosal glands and stroma. The liver parenchyma revealed a patchy but diffuse degeneration, haphazard in its distribution and varying in severity from cloudy swelling to actual necrosis. The lobular pattern was disturbed in the areas where cords of liver cells were broken up or irregular segments of parenchyma were shrunken. The extreme engorgement of the sinusoids and the presence of many new and recent hemorrhages suggested that much of the pigment present was of hemic origin. The adrenal glands were congested, with focally hemorrhagic medullae. Depletion of cytoplasm in the cortical cells was fairly prominent. The kidneys were involved by a severe tubular nephrosis. Extensive degeneration and necrosis of the tubular epithelium were found, with no particular regard for any upper or lower nephron localization. The tubular lumens contained desquamated lining cells and irregular clumps and granules of amorphous material.

The presence of granular brown pigment in many of the tissues studied aroused the suspicion that the material might represent the toxic agent. In order to distinguish this material from any breakdown product of hemoglobin, tissue sections were washed to remove formaldehyde fixation pigment and stained by the Turnbull blue method for reduced iron. By this means, iron was demonstrated in the gastric and intestinal mucosa. It had impregnated the surface epithelium; it was present in the lumens of the glands and in the small veins, often appearing concentrated in the endothelium. The rather large amounts of pigment in the liver were apparently of hemic origin or else represented some altered product of the absorbed metal; no reduced iron was found in the liver. The kidney sections, stained by the Turnbull method, revealed numerous tubular casts of the characteristic blue color indicative of reduced iron.

COMMENT

The case reported here illustrates many features common to other reported cases of acute iron intoxication.[1] The patient is usually a young child who has accidentally ingested a large amount of some medicinal iron preparation. In a few hours he appears quite ill, pale, restless, and nauseated. Vomiting and diarrhea are common but not invariable. Gradually the child becomes drowsy and lethargic, often lapsing into semiconsciousness or coma. Signs of peripheral circulatory failure then intervene: a falling blood pressure, rapid pulse, and cool, cyanotic skin. The first "danger period" is this early phase of about four to six hours, when the patient may die in an apparent state of shock. Often, however, the child seems to improve rapidly during the next 12 to 24 hours, only to die suddenly during the second danger period, 24 to 48 hours after the ingestion of the iron.[2] At autopsy, the most striking lesions are seen in the gastrointestinal tract, where the escharotic action of the metal has produced mucosal necrosis, congestion, and focal hemorrhages in the stomach and small intestine. Diffuse congestion and petechial hemorrhages of the other viscera are usually present. Hepatic and renal lesions are variable. Mild degenerative changes (cloudy swelling) are described. Rarely, more severe lesions such as focal necrosis are found in the liver.[3]

Approximately one-half of patients ill enough to attract attention as examples of iron intoxication have recovered. Clinical studies of ill and convalescing patients are rather meager. Spencer [2] reports that the serum iron may rise to 15 to 100 times its normal level. Abnormal results were obtained when liver function tests were performed on several recently recovered patients. Murphy and co-workers,[3] however, were unable to find evidence of hepatic or renal damage in their case. In our case, both the liver and the kidneys showed unusually severe degenerative changes.

The mechanism of action of iron as a toxic agent is unknown. Most investigators are in agreement that the early symptoms of vomiting, diarrhea, and dehydration are due to the corrosive action on the gastrointestinal mucosa, leading to ulceration, edema, and loss of fluid into the gastrointestinal tract. Furthermore, this destruction doubtless abolishes the normal "mucosal block" that ordinarily inhibits the absorption of more than small amounts of iron.[1a] One may postulate that the loss of body fluid via the gastrointestinal tract can produce severe dehydration and irreversible shock in much the same manner as occurs in infant diarrheas. Against this hypothesis it can be argued that in none of the reported cases was the loss of fluid by diarrhea and vomiting considered to be impressive. Also many of the patients were old enough so that such fluid loss as occurred might be expected to be borne with greater impunity. Finally, prompt and energetic efforts to restore the circulating volume were often unsuccessful.

That the basic picture of acute iron intoxication is essentially the same as peripheral circulatory collapse is now widely accepted. As pointed out by J. P. Smith,[4] the cold, cyanotic skin, rapid pulse, and irregular respirations are typical. Restlessness, drowsiness, and coma had earlier led some of the English investigators to propose a specific toxic effect of iron on the central nervous system.[1c] It should be borne in mind, however, that the same symptoms are common in impending shock from any cause. Autopsy findings in the central nervous system in the cases reported to date have yielded nothing conclusive. The greatest interest has been stimulated by the work linking iron intoxication to the vasodilator material (VDM) shown to be present in the blood of animals in experimental shock. This substance, normally present in liver and spleen, and capable of producing shock when released into the blood stream, is now believed to be identical with ferritin.[4] The latter, a com-

1. (*a*) Smith, R. P.; Jones, C. W., and Cochran, W. E.: Ferrous Sulfate Toxicity: Report of Fatal Case, New England J. Med. **243**: 641-645 (Oct. 26) 1950. (*b*) Poisoning from Accidental Ingestion of Medicinal Iron, editorial, J. A. M. A. **148**: 1280 (April 12) 1952. (*c*) Duffy, T. L., and Diehl, A.: Ferrous Sulfate Poisoning: Report of Three Cases, J. Pediat. **40**: 1-5 (Jan.) 1952. (*d*) Swift, S. C.; Cefalu, V., and Rubell, E. B.: Ferrous Sulfate Poisoning: Report of Fatal Case, ibid. **40**: 6-10 (Jan.) 1952. (*e*) Forbes, G.: Poisoning with Preparation of Iron, Copper, and Manganese, Brit. M. J. **1**: 367-370 (March 22) 1947.

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pound of iron with the protein apoferritin, represents a normal storage form of iron and also the immediate product of iron absorption in the intestinal mucosa. It is postulated that excess absorption of iron leads to excess formation of ferritin, resulting in severe and prolonged shock.[4] The diffuse and nonspecific nature of the anatomic lesions is not particularly helpful in elucidating the mechanism of iron toxicity. Nevertheless, the histological findings in these organs are consistent with those produced by anoxia secondary to peripheral circulatory collapse, except, of course, for the presence of iron in the renal tubules. In regard to this latter finding, the situation is analagous to that in the "crush" syndrome and the hemoglobinuric nephroses. The tubular casts may possibly come to be considered as incidental rather than as a direct causative feature.

33-37 Harrison St. (Dr. Kosinski).

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83: 65-72, 1962

THE ACUTE TOXICITY OF FERROUS SALTS ADMINISTERED TO DOGS BY MOUTH

P. F. D'Arcy and E. M. Howard

Research Division, Allen and Hanburys Limited, Ware, Hertfordshire

(Plates XXV and XXVI)

The efficacy of iron therapy in the treatment of certain anæmias has long been recognised. The physicians, Sydenham (1681, translated 1850) and Blaud (1832) prescribed large doses of iron and obtained beneficial results. Later workers (Quincke, 1895; von Noorden, 1906) advocated the use of much smaller doses because of the limited absorption of iron from the intestine; these small doses proved ineffective clinically and the therapeutic value of iron became discredited. Later investigators (Lichtenstein, 1918; Meulengracht, 1923; Brock and Hunter, 1937; Widdowson and McCance, 1937) re-emphasised the need for large doses of iron and showed that ferrous salts were more effective than ferric (Witts, 1936).

However, with the increased use of large doses of ferrous salts, there has been a progressive increase in the number of fatalities from accidental ingestion of large amounts of the drugs, especially amongst children. Seventeen deaths were recorded in Great Britain between 1940 and 1949, and 32 between 1950 and 1953 (Committee on Toxicology, Report, 1959). The symptoms of this poisoning have been reported as shock, gastro-intestinal irritation, necrotic lesions of the stomach and intestine, together with slight damage to other organs, especially the liver and heart (Forbes, 1947; Thomson, 1947, 1950; Duffy and Diehl, 1952; Swift, Cefalu and Rubell, 1952). Recently Davis (1960) has reported a case of gastric stricture in a girl aged 6 months, which developed 6 weeks after the ingestion of 30 tablets of ferrous sulphate; there were dense perigastric adhesions, a gastric stricture 5 cm. long, thickening and rigidity of the stomach, and extensive ulceration of the gastric mucosa. Nine other cases of gastric stricture which occurred approximately 5 weeks after the ingestion of large numbers of ferrous sulphate tablets were also reported. Furthermore, many cases of intolerance to ferrous salts occur, with symptoms of nausea, vomiting and gastro-intestinal upsets (Benstead and Theobald, 1952; Gatenby and Lillie, 1955; O'Sullivan, Higgins and Wilkinson, 1955).

Because of the present widespread clinical use of a variety of iron preparations, and the frequency of accidental poisonings, it was thought of importance to determine the relative safety of these iron salts when administered orally. The dog was selected as the experimental animal for this work, because in this species it is possible to observe many of the toxic symptoms that have been reported in man.

Materials and methods

Mongrel dogs of either sex weighing 6–14 kg. were used; they were housed in groups of 6, and maintained on a proprietary brand of dog food, dog biscuits and

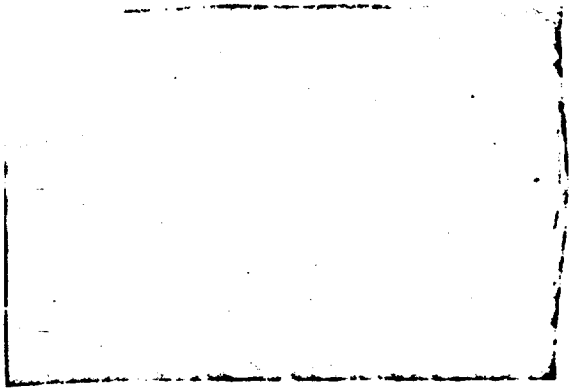

FIG. 1.—Dog's stomach 24 hr after oral administration of ferrous carbonate tablets (3·0 g. ferrous iron per kg. body-weight).

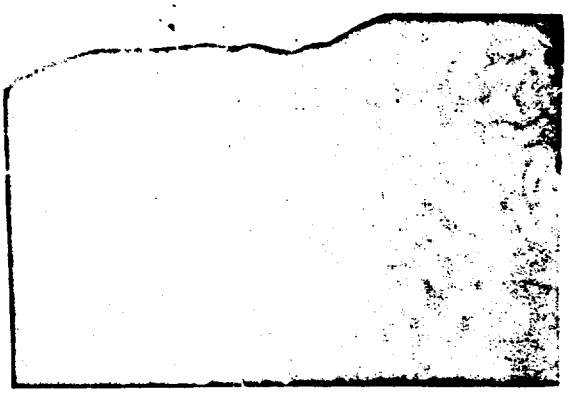

FIG. 2.—Dog's stomach 24 hr after oral administration of ferrous carbonate tablets (1·5 g. ferrous iron per kg. body-weight).

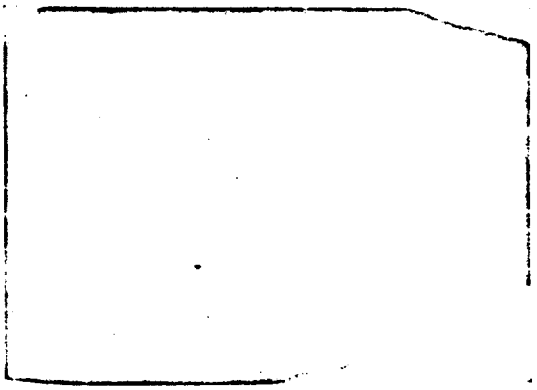

FIG. 3.—Dog's stomach 24 hr after oral administration of ferrous sulphate tablets (0·75 g. ferrous iron per kg. body-weight).

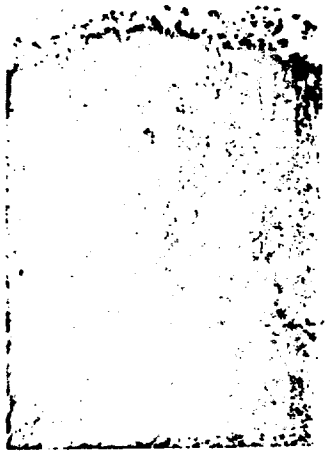

FIG. 4.—Section of dog's stomach showing infiltration of iron into the submucosa 24 hr after oral administration of ferrous sulphate (0·6 g. ferrous iron per kg.). Perls' prussian blue. ×15.

TOXICITY OF FERROUS SALTS IN DOGS

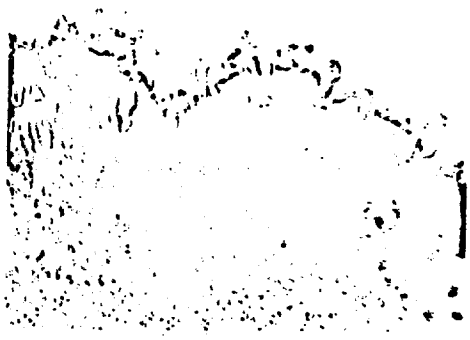

FIG. 5.—Section of dog's stomach showing infiltration of iron and epithelial damage, 24 hr after oral administration of ferrous sulphate (0·6 g. ferrous iron per kg.). Perls' prussian blue. ×30.

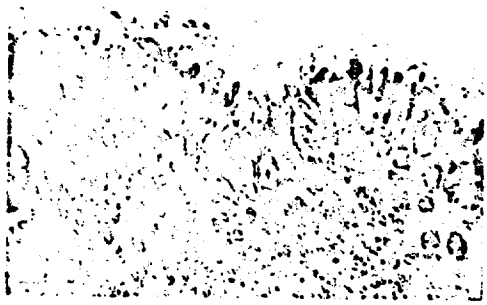

FIG. 6.—Section of dog's stomach showing damage to epithelium 24 hr after oral administration of ferrous sulphate (0·6 g. ferrous iron per kg.). Haematoxylin and eosin. ×30.

FIG. 7.—Section of duodenum from dog, 24 hr after oral administration of ferrous sulphate (0·6 g. ferrous iron per kg.), showing infiltration of iron into epithelial cells of villi. Perls' prussian blue. ×28.

FIG. [illegible] Section of dog's stomach [illegible]g intact epithelium and [illegible]e of iron infiltration 24 hr [illegible]oral administration of [illegible] carbonate (1·5 g. ferrous [illegible] per kg.). Perls' prussian [illegible] ×28.

tap water. They were fed once a day and this routine was maintained throughout the experiments. The ferrous salts were tested in two forms :—(*a*) the chemical compressed into pellets, and (*b*) the corresponding commercial preparation of the drug in tablet form. The pellets or tablets were administered by placing them at the back of the throat and inducing the dog to swallow. The animals were killed by an intravenous or intrathoracic injection of pentobarbitone sodium (Nembutal) 24 hr after dosing ; necropsies were carried out immediately. All organs and tissues were examined and portions of heart, lung, liver, kidney, spleen, stomach and intestine were removed for histological examination. Sections of these organs were stained with haematoxylin and eosin to detect cellular damage, and duplicate sections were stained by Perls' method to determine the degree of iron absorption or iron deposition in the tissues.

Because of the large numbers of tablets involved, it was generally not possible to administer sufficient quantities of the iron compounds to cause death, therefore the toxic effects of the compounds were assessed from the severity of the symptoms and of the macroscopic and microscopic changes in the organs.

Results

In initial experiments the relative toxicity of ferrous carbonate, sulphate and gluconate was investigated ; for convenience, these compounds were administered compressed into pellets. A dose of ferrous carbonate as high as 1·5 g. ferrous iron per kg. did not produce any symptoms of toxicity, nor was there any post-mortem or histological evidence of damage to the stomach and intestine. In contrast with this, ferrous sulphate at a dose level of 0·6 g. ferrous iron per kg. was fatal, and a dose of 0·3 g. per kg. produced extensive ulceration and inflammation of the stomach and duodenum. Ferrous gluconate, although less toxic than the sulphate, was more toxic than the carbonate, since, although not lethal, it produced gastro-intestinal damage at dose levels of 0·75 and 0·375 g. iron per kg. The results of these experiments are summarised in table I.

These results made it seem worthwhile to do some experiments in which accidental ingestion of large numbers of tablets of ferrous salts was simulated ; commercial preparations of ferrous carbonate, sulphate, gluconate and succinate were used. Ferrous carbonate tablets contain about 50 mg. ferrous iron per tablet ; ferrous sulphate (B.P. and N.F.), 60 mg. ; ferrous gluconate B.P., 39 ; and ferrous succinate, 37 mg. The results of these experiments are summarised in table II, and confirm the results obtained in the initial experiments.

At a dose level of 3·0 g. ferrous iron per kg., ferrous carbonate produced slight ulceration at the junction of the fundus and pylorus (dog 8, fig. 1), or slight inflammation of the fundus. At 1·5 g. per kg., however, there was no macroscopic or histological evidence of any damage to the stomach or intestine (dog 11, fig. 2). Ferrous sulphate caused severe ulceration of the fundus, pylorus and gastroduodenal junction when administered at doses of 0·75 and 0·375 g. per kg. (dog 22, fig. 3). Decreased dosage caused a correspondingly less degree of damage, but even at doses as low as 0·012–0·023 g. per kg. there were isolated patches of ulceration. Ferrous gluconate and ferrous

TABLE I

Toxic effects in dogs of oral administration of ferrous salts

Dog	Body-weight (kg.)	Compound	Dose (g. ferrous iron per kg. body-weight)	General condition after administration of drug	Occurrence of			Post-mortem evidence of gastro-intestinal damage in					
					vomiting	malaise	death	fundus	pylorus	gastro-duodenal junction	duodenum	mid intestine	ileum
1	12·5	Ferrous carbonate	1·5	Normal	—	—	—	—	—	—	—	—	—
2	8·4	Ferrous carbonate	1·5	Normal	—	—	—	—	—	—	—	—	—
3	9·0	Ferrous carbonate	1·0	Normal	—	—	—	—	—	—	—	—	—
4	9·7	Ferrous sulphate	0·6	Severe diarrhœa present	+	+	+	+ + +	+ + +	+ + +	+ +	+ +	+ +
5	11·0	Ferrous sulphate	0·3	Diarrhœa present	+	—	—	+ +	+ +	+	±	—	—
6	10·5	Ferrous gluconate	0·75	Normal	+	—	—	+ + +	+ + +	+ + +	+	±	±
7	15·8	Ferrous gluconate	0·375	Normal	+	—	—	+	+	+	—	—	—

For vomiting, malaise or death, + = occurred. For post-mortem evidence of gastro-intestinal damage, + + + = severe ulceration; + + = ulceration; + = inflammation, isolated areas of ulceration, or both; ± = slight inflammation; and — = no evidence of damage.

succinate were less toxic than ferrous sulphate but more toxic than ferrous carbonate, the former causing slight ulceration of the fundus at a dose level of 0·094 g. per kg. and the latter producing inflammation of the gastroduodenal junction at a level of 0·187 g. per kg.

Microscopical examination of sections of the fundus and pylorus, from dogs that had received toxic doses of ferrous sulphate, showed the presence of iron in the columnar epithelium, extending down to the submucosa; there was necrosis of the epithelial cells, and haemorrhage in the submucosa, which corresponded to the areas of iron deposition (figs. 4–6). In some dogs there were traces of iron in the epithelial lining of the duodenum, jejunum, ileum and colon, with corresponding breakdown of the epithelial cells (fig. 7). Similar histological changes were observed in the sections from animals treated with 0·375 g. per kg. ferrous gluconate, 0·75 g. per kg. ferrous succinate, or with 3·0 g. per kg. ferrous carbonate. However, histological sections from dogs that had received 1·5 g. per kg. ferrous carbonate appeared normal (fig. 8). Microscopical sections of the spleens of all dogs treated with high doses of a ferrous compound showed heavy iron deposition. Sections from other organs were normal in histology, but in some dogs there were also isolated patches of iron deposition in the lungs or liver.

Evidently ferrous carbonate at oral dose levels of 1·5 g. ferrous iron per kg. produces no toxic symptoms, but ferrous sulphate and ferrous sulphate compound are toxic at levels as low as 0·012 g. per kg.; ferrous gluconate is non-toxic at 0·047 g. per kg. and ferrous succinate at 0·094 g. per kg.

Initial studies have been performed on the chronic toxicities of ferrous sulphate and ferrous carbonate, in which the chemical salt in powdered form was administered daily for 14 consecutive days. Results again indicated that ferrous carbonate was far less toxic than ferrous sulphate.

DISCUSSION

It is apparent from these results, both with the iron salts and with the commercial tablet preparations, that ferrous carbonate is the least toxic of the compounds tested; it produces no toxic symptoms whatsoever at a dose level at which ferrous sulphate is fatal in some dogs and causes severe retching and vomiting, and gross ulceration of the fundus, pylorus, and gastroduodenal junction in others. The histological evidence confirms this. Ferrous sulphate, alone or in compound tablets, causes some damage at dose levels as low as 0·012–0·023 g. per kg.; this dose is the equivalent of about 4 tablets. The slight inflammation or ulceration caused by this very small number of tablets may help to explain the intolerance to ferrous sulphate so frequently reported. Reports of accidental death due to iron poisoning show that in some cases as many as 30–40 tablets are ingested by children of less than 2 yr of age; this represents an approximate dose level of 0·18–0·24 g. ferrous iron per kg. for a child weighing 10 kg.

TABLE II

Toxic effects in dogs of oral administration of iron tablets

Dog	Body-weight (kg.)	Compound	Dose (g. ferrous iron per kg. body-weight)	No. of tablets administered	General condition after administration of drug	Occurrence of			Post-mortem evidence of gastro-intestinal dam					
						vomiting	malaise	death	fundus	pylorus	gastro-duodenal junction	duodenum	mid-intestine	Ileum
8	14·3	Ferrous carbonate	3·0	800	Normal	−	−	−	+	−	−	−	−	−
9	10·9	" "	3·0	654	"	−	−	−	±	−	−	−	−	−
10	9·1	" "	2·4	441	"	−	−	−	±	−	−	−	−	−
11	13·6	" "	1·5	408	"	−	−	−	−	−	−	−	±	±
12	10·7	" "	1·5	321	"	−	−	−	−	−	−	−	−	−
13	10·0	" "	1·5	300	"	−	−	−	−	−	−	−	−	−
14	8·4	" "	1·5	252	"	−	−	−	−	−	−	−	−	−
15	12·0	" "	0·75	173	"	−	−	−	−	−	−	−	−	−
16	8·3	" "	0·75	125	"	−	−	−	−	−	−	−	−	−
17	8·2	" "	0·75	123	"	−	−	−	−	−	−	−	−	−
18	7·7	" "	0·75	116	"	−	−	−	−	−	−	−	−	−
19	14·8	Ferrous sulphate (B.P.)	0·75	184	"	+	−	−	+++	+++	+++	±	−	−
20	9·0													
21	7·3	" "	0·75	112	"	+	−	−	++	++	++	−	−	−
22	6·3	" "	0·75	92	"	+	−	−	+++	+++	+++	−	−	−
23	12·0	" "	0·75	80	Poor	+	+	−	+++	+++	+++	−	−	−
24	9·6	" "	0·375	75	Normal	−	−	−	+	+	+	−	−	−
25	8·1	" "	0·375	60	"	−	−	−	++	++	++	+	−	−
26	11·5	" "	0·375	51	"	+	−	−	+++	+	+	−	−	−
27	7·3	" "	0·187	36	"	−	−	−	−	+	+	−	−	−
28	9·8	" "	0·187	23	"	+	−	−	++	++	++	++	−	−
29	9·5	" "	0·094	15	"	+	−	−	−	−	+	−	−	−
30	10·9	" "	0·094	15	"	+	−	−	±	±	−	+	−	−
31	10·5	" "	0·047	9	"	−	−	−	±	+	±	−	−	−
32	7·9	" "	0·047	8	"	−	−	−	±	−	−	−	−	−
33	8·8	" "	0·023	3	"	−	−	−	−	+	−	−	−	−
34	8·0	" "	0·023	4	"	−	−	−	−	−	±	−	−	−
35	8·0	" "	0·012	2	"	−	−	−	−	+	−	−	−	−
			0·012	2	"	−	−	−	−	−	−	−	−	−
36	8·6	Ferrous sulphate compound	1·5	199	"	+	−	−	+++	+++	++	+	+	±
37	7·9	" "	0·75	92	"	++	−	−	+++	+++	+++	−	−	−
38	11·0	" "	0·375	64	"	+	−	−	+	+	+	+	±	−
39	8·9	" "	0·187	26	"	−	−	−	−	+	−	−	−	−
40	8·2	" "	0·187	24	"	−	−	−	+	−	−	−	−	−
41	9·3	" "	0·047	7	"	−	−	−	+	+	+	−	−	−
42	8·2	" "	0·047	6	"	−	−	−	+	±	−	±	−	−
43	7·3	" "	0·023	3	"	−	−	−	+	±	−	−	−	−
44	7·7	" "	0·023	3	"	−	−	−	±	−	−	−	−	−
45	9·6	" "	0·012	2	"	−	−	−	−	±	−	−	−	−
46	8·1	" "	0·012	1·5	"	−	−	−	−	−	−	−	−	−
47	7·7	Ferrous gluconate	1·5	296	"	+	−	−	++	++	++	+	±	±
48	7·3	" "	1·5	281	"	+	−	−	+	+	−	−	−	−
49	10·5	" "	0·75	206	"	−	−	−	++	++	++	−	−	−
50	10·0	" "	0·75	197	"	−	−	−	+	+	−	−	−	−
51	11·25	" "	0·44	127	"	+	−	−	+	−	−	−	−	−
52	8·9	" "	0·375	86	Severe immediate diarrhœa	−	−	−	−	−	−	−	−	−
53	10·0	" "	0·187	49	Normal	−	−	−	±	±	−	−	−	−
54	9·1	" "	0·187	45	"	−	−	−	±	±	−	−	−	−
55	8·9	" "	0·094	22	"	−	−	−	±	±	−	−	−	−
56	7·3	" "	0·094	18	"	−	−	−	±	±	−	−	−	−
57	11·1	" "	0·047	14	"	−	−	−	±	±	−	±	−	−
58	8·2	" "	0·047	10	"	−	−	−	−	−	−	−	−	−
59	10·0	Ferrous succinate	1·5	406	"	−	−	−	++	++	+	−	−	−
60	9·3	" "	0·75	189	"	−	−	−	+	+	−	−	−	−
61	6·8	" "	0·75	138	"	−	−	−	−	−	+	−	−	−
62	11·4	" "	0·375	116	"	−	−	−	+	−	+	−	−	−
63	13·2	" "	0·187	67	"	−	−	−	−	−	±	−	−	−
64	7·3	" "	0·094	18	"	−	−	−	−	−	−	−	−	−
65	6·1	" "	0·094	15	"	−	−	−	−	−	−	−	−	−

Symbols as in table I.

It is obviously not possible to relate the dose levels that produce fatalities in children to those that cause ulceration in dogs' stomachs, but similar doses of ferrous sulphate in dogs cause widespread ulceration of the fundus, pylorus and gastroduodenal junction. This degree of ulceration is not produced by ferrous carbonate even at a dose level as high as 3·0 g. ferrous iron per kg., a level which would represent the ingestion of more than 600 tablets of ferrous carbonate by a 2-yr-old child.

Somers (1947) on comparing the median lethal doses of ferrous sulphate, ferrous sulphate compound, ferrous gluconate, ferrous carbonate, ferric chloride, and ferric and ammonium citrate, also found that ferrous carbonate was less toxic than the other compounds tested; he attributed this to the relative insolubility of the carbonate in gastric and intestinal contents, and its consequent poor absorption. However, clinical trials with the preparation of ferrous carbonate used for these experiments have shown that at dose levels similar to those for ferrous sulphate it effectively raises the hæmoglobin levels in cases of iron-deficiency anæmia.

It is also of interest to note that Somers (1947) reported that the toxicity of ferrous sulphate was reduced by the simultaneous administration of sodium bicarbonate. From the histological evidence of the present series of experiments, however, it appears that the cellular damage is related to the presence of the iron itself, but it may be that the increased localised gastric acidity produced by the administration of some iron compounds initiates damage to the stomach mucosa, which then enables the iron to infiltrate into the tissues, with consequent necrosis and hæmorrhage. Whether this damage in itself is the cause of death, or whether death is caused by an abnormal systemic absorption of iron from the stomach through the damaged tissues, it is apparent that it is preferable to use an iron preparation that produces little or no initial damage to the stomach and intestinal mucosa.

Summary

In dogs given ferrous salts, either as pure salt or as equivalent commercial preparations by mouth, ferrous carbonate was considerably less toxic than ferrous sulphate, ferrous gluconate or ferrous succinate.

Histological examination of sections of the stomachs and intestines of dogs given ferrous salts by mouth showed that the tissue damage was related to the presence of iron in the cells.

It is suggested that preferential use of the less toxic ferrous carbonate in the treatment of iron-deficiency anæmias would help to prevent the increasing number of deaths among children that are due to the accidental ingestion of large numbers of iron tablets, and would also help some of the cases of intolerance to iron salts.

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FERROUS CARBONATE: TOXICITY AND EFFECT ON HAEMOGLOBIN LEVELS IN EXPERIMENTAL IRON-DEFICIENCY ANAEMIA

P. F. D'ARCY * AND ELSIE M. HOWARD *
Research Division, Allen and Hanburys Limited, Ware, Hertfordshire

THE present widespread clinical use of a variety of oral iron preparations has been the indirect cause of an increasing number of accidental poisonings in young children (Committee on Toxicology, Report, 1959; D'Arcy and Howard, 1962*a*). In a previous publication, the acute toxicities of ferrous salts administered orally to dogs were compared, and ferrous carbonate was shown to be the least toxic of the compounds investigated (D'Arcy and Howard, 1962*b*). In the present study, ferrous carbonate was examined for subacute toxicity and, because it had been suggested that the lack of toxicity shown in the acute experiments was due to poor absorption, the ferrous carbonate preparation was also examined for its effect on haemoglobin levels in experimental iron-deficiency anaemia. The dog was selected as the experimental animal for toxicity studies because, in this species, it is possible to produce many of the toxic symptoms that have been reported in man. The pig was selected for the haemoglobin studies because piglet anaemia is a simple iron-deficiency condition that can easily be produced experimentally.

MATERIALS AND METHODS

Subacute toxicities

Mongrel dogs of either sex weighing 5–16 kg. were used; they were housed in groups of 6, and maintained on a proprietary brand of dog food, dog biscuits and tap water. They were fed once a day and this routine was maintained throughout the experiments. The ferrous salts were tested as the corresponding commercial preparation of the drug in tablet form, and were administered by placing them at the back of the throat and inducing the animal to swallow. The dogs were dosed daily for 14 consecutive days, and killed 24 hr after the final dose with an intravenous or intrathoracic injection of pentobarbitone sodium (Nembutal). Necropsies were carried out immediately; all organs and tissues were examined and portions of heart, lung, liver, kidney, spleen, stomach and intestine were removed for histological examination.

Because of the large numbers of tablets involved, it was generally not possible to administer sufficient quantities of the iron compound to cause death; the toxic effects of the compounds were therefore assessed from the severity of the symptoms and from the macroscopic and microscopic changes in the organs.

Effect of ferrous carbonate on iron-deficiency anaemia

Experimental iron-deficiency was induced in piglets, and the commercial preparation of ferrous carbonate was examined for its effect on the haemoglobin levels of

* Present address of authors: Department of Pharmacology, Faculty of Pharmacy, University of Khartoum, Sudan.

these animals. Two Wessex saddleback gilts were housed indoors on concrete runs for 1–2 wk prior to farrowing; one gilt produced a litter of 10 live piglets, the other a litter of 12 piglets. These were also kept on concrete runs throughout the experiment; they were suckled until the 3rd wk, and from then onwards they also received a special creep feed, deficient in iron. This contained coarse ground wheat, 20 per cent.; barley meal, 20 per cent.; maize meal, 23·3 per cent.; middlings, 10 per cent.; fish meal, 7·5 per cent.; soya meal, 7·5 per cent.; groundnut meal, 5 per cent.; ground limestone, 1·25 per cent.; sodium chloride, 0·22 per cent.; flaked maize, 5 per cent.; and Vitablend 605 (Glaxo) 0·22 per cent.; total iron, 2–4 p.p.m.

The piglets were bled from the ear vein on the first day after birth and then on alternate days until the 17th day; subsequently blood samples were taken at specific intervals until the 49th day, when the study was terminated. Haemoglobin was estimated colorimetrically. Dosage with ferrous carbonate tablets was commenced on the 7th day after birth, by which time the piglets had developed clinical iron-deficiency anaemia; treatment was continued for 15 days. The piglets were weighed at regular intervals throughout the experiment.

Results

Subacute toxicity

The subacute toxicity of a commercial preparation of ferrous carbonate was assessed and compared with those of the more commonly used preparations, ferrous sulphate compound and ferrous gluconate (table I). The amount of ferrous iron contained in each tablet of the preparation used was: ferrous carbonate, 50 mg.; ferrous sulphate compound, 60 mg.; and ferrous gluconate B.P., 39 mg. With a single exception, doses of 1·0, 0·5, and 0·25 g. ferrous iron per kg. daily in the form of ferrous carbonate did not produce any symptoms of toxicity, nor was there any post-mortem or histological evidence of damage to the stomach or intestine. The exception was one dog (no. 68), which received 0·5 g. ferrous iron per kg. daily, in the stomach of which there was evidence of some inflammation of the fundus and slight inflammation in the pylorus. In contrast to the results obtained with ferrous carbonate, ferrous sulphate compound was toxic at dose levels of 0·1 g., 0·05 g. and 0·025 g. ferrous iron per kg. daily, and non-toxic at a dose level of 0·005 g. ferrous iron per kg. Ferrous gluconate did not show any evidence of gross toxicity, although a dose of 0·5 g. ferrous iron per kg. daily caused slight inflammation of the fundus and pylorus regions of the stomach, and in one of the dogs (no. 80) there was also evidence of slight inflammation of the mid-intestine. Doses of 0·25 g. ferrous iron per kg. daily, or less, were without toxic effect. Microscopic examination of sections of the stomach and intestine from dogs that had received toxic doses of iron salts showed lesions similar to those described in the previous study (D'Arcy and Howard, 1962*b*); tissue damage was related to the presence of iron in the cells. As in those acute toxicity studies, the spleens of all dogs treated with high doses of iron preparations showed heavy deposition of iron. Sections from other organs were histologically normal, but in some dogs there

TABLE I

Toxic effects in dogs of subacute oral administration of commercial preparations of ferrous salts

Dog no.	Body weight (kg.)	Compound used	Dose (g. ferrous iron per kg. body weight)	General condition of dog during experiment	Weight gain or loss (kg.) during experiment	Occurrence of			Post-mortem evidence of gastro-intestinal damage in						
						vomiting	malaise	death	fundus	pylorus	gastro-duodenal junction	duodenum	mid-intestine	ileum	large intestine
66	8·3	Ferrous carbonate	1·0	Normal	+0·5	−	−	−	−	−	−	−	−	−	−
67	6·8	" "	1·0	Normal	−0·2	−	−	−	−	−	−	−	−	−	−
68	11·7	" "	0·5	Normal	No change	−	−	−	−	−	−	−	−	−	−
69	7·5	" "	0·5	Normal	−0·6	−	−	−	+	±	−	−	−	−	−
70	9·7	" "	0·5	Normal	−0·4	−	−	−	−	−	−	−	−	−	−
71	6·6	" "	0·25	Normal	+1·3	−	−	−	−	−	−	−	−	−	−
72	5·7	" "	0·25	Normal	+1·0	−	−	−	−	−	−	−	−	−	−
73	5·5	" "	0·125	Normal	No change	−	−	−	−	−	−	−	−	−	−
74	9·5	Ferrous sulphate compound	0·1	Normal	+0·3	−	−	−	++	−	−	−	−	−	−
75	10·5	" " "	0·05	Normal	+0·7	+	−	−	−	+	−	−	−	−	−
76	11·7	" " "	0·05	Normal	No change	−	−	−	+	++	−	−	−	−	−
77	13·4	" " "	0·025	Normal	−0·6	−	−	−	+	++	+	−	−	−	−
78	15·5	" " "	0·025	Normal	+3·4	+	−	−	+	+	−	+	−	−	−
79	8·6	" " "	0·005	Normal	−0·1	−	−	−	−	−	−	−	−	−	−
80	8·0	Ferrous gluconate	0·5	Copious salivation	+0·2	−	−	−	±	±	−	−	±	−	−
81	5·7	" "	0·5	"	+0·1	−	−	−	±	±	−	−	−	−	−
82	7·7	" "	0·25	Normal	+0·1	+	−	−	±	±	−	−	−	−	−
83	9·3	" "	0·125	Normal	+1·0	−	−	−	−	−	−	−	−	−	−
84	6·7	" "	0·063	Normal	+0·1	−	−	−	−	−	−	−	−	−	−
85	8·6	" "	0·015	Normal	+0·3	−	−	−	−	−	−	−	−	−	−

For vomiting, malaise or death, + = occurred. For post-mortem evidence of gastro-intestinal damage, +++ = severe ulceration; ++ = ulceration; + = inflammation, isolated areas of ulceration, or both; ± = slight inflammation; and − = no evidence of damage.

In table II, comparison is made between the present results and the maximum non-toxic levels of the iron salts tested in the earlier acute studies (D'Arcy and Howard, 1962*b*).

TABLE II

*Comparison between the maximum non-toxic doses of commercial preparations of ferrous salts, administered orally to dogs in acute * and subacute tests*

Compound	Maximum non-toxic dose (g. ferrous iron per kg. body weight) in	
	acute tests *	subacute tests
Ferrous carbonate	1·5	>1·0
Ferrous gluconate	0·05	0·25
Ferrous sulphate compound	<0·012	<0·025, >0·005

* D'Arcy and Howard (1962*b*).

Effect of ferrous carbonate on iron-deficiency anaemia

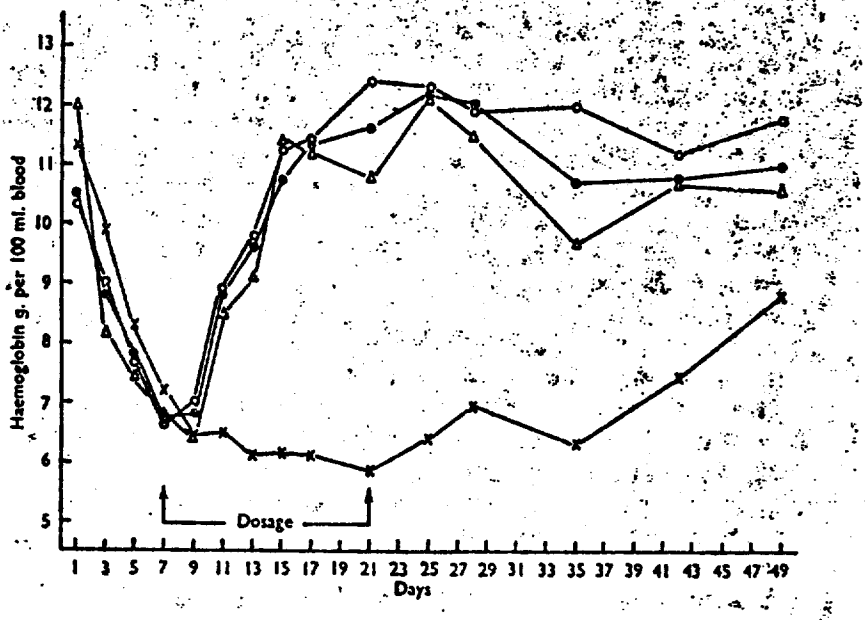
The 22 piglets in the two litters were divided into 4 groups; one group of 6 pigs received 200 mg. ferrous iron per piglet daily (4 tablets); the second group received 50 mg. per piglet daily (1 tablet), and a third group of 3 pigs received 50 mg. on alternate days; the remaining 7 piglets did not receive any treatment and were used as controls. It was necessary in this experiment to dose per piglet rather than by body weight, since the latter method would have necessitated the difficult division of sugar-coated tablets into accurate fractions.

Haemoglobin levels in all the piglets fell to approximately 60 per cent. of their birth levels within 7 days. Oral administration of ferrous carbonate caused an appreciable rise of the lowered haemoglobin levels within 3 days, and within 8 days the treated piglets had attained their birth haemoglobin levels. There was no appreciable difference between the effect of 50 mg. ferrous iron per piglet on alternate days and the effects of the higher doses. In contrast to this, the untreated piglets showed a typical picture of piglet anaemia, with the usual partial recovery commencing during the 6th wk after birth; these results are shown graphically in the figure. There was no apparent difference between the mean body weights of the treated and control piglets throughout the experiment.

DISCUSSION

The maximum non-toxic levels of ferrous carbonate and ferrous sulphate shown in the present series of subacute toxicity tests and the earlier acute tests are very similar (table II). Ferrous gluconate,

however, appears to be less toxic when administered subacutely than when a single dose was given. There is approximately a six-fold difference; this would suggest that the dogs develop some degree of tolerance to the toxic effects of this iron preparation, and that the ulceration or inflammation produced by the initial doses was healed by the end of the experiment. Dogs receiving ferrous gluconate salivated copiously after the second or third dosing, and the degree of salivation progressively increased during the course of the experiment, until by the tenth day the dogs had developed a conditioned reflex and salivated when brought from their kennels to the treatment room. Preliminary



FIGURE—The effect of oral administration of a commercial preparation of ferrous carbonate on the haemoglobin levels of iron-deficient piglets. Arrows indicate duration of iron administration; ○ 200 mg. ferrous iron per piglet daily (6 pigs). ● 50 mg. ferrous iron per piglet daily (6 pigs). △ 50 mg. ferrous iron per piglet on alternate days (3 pigs). × Untreated controls (7 pigs).

experiments indicated that the salivation was not due to the ferrous gluconate but to the coloured coating of the commercial tablets. Post-mortem examination of these dogs revealed a thick and copious mucoid secretion in the stomach and small intestine, and it is thought that this may have had a dual effect: firstly, in preventing fresh areas of ulceration and inflammation from developing after subsequent dosing, and secondly, in covering the damaged tissues resulting from the initial doses of the iron tablets, and thereby assisting in the healing process.

Somers (1947) found that ferrous carbonate was less toxic than a variety of other iron compounds when tested orally in small laboratory animals. He attributed this to the relative insolubility of the carbonate

in gastric and intestinal contents, and its consequent poor absorption. However, the present studies with ferrous carbonate in the treatment of piglet anaemia have shown that the haemoglobin levels are effectively raised by doses that are similar, on a body-weight basis, to doses of ferrous iron used clinically. Since the carbonate, at such dose levels, is sufficiently well absorbed to raise the haemoglobin levels effectively, it appears that its lack of toxicity cannot be attributed to its poor absorption.

Sharp (1962) has suggested that the greater toxicity of the sulphate as compared with the carbonate or gluconate may be due to the liberation of sulphuric acid into the surrounding tissues. Since iron has a low electronegativity value (1·7–1·8), its salts are poorly ionised and are largely hydrolysed in solution; it follows, therefore, that they are more readily transported across the cell membranes than the corresponding free acid, but readily liberate free acid into the tissues. Sharp quotes, as an example, that 20×5 gr. tablets of ferrous sulphate are equivalent to about 10 fluid drachms of dilute sulphuric acid and are probably far more dangerous, because free sulphuric acid, being highly ionised, is not so readily, if at all, absorbed from the gut. This theory, based on chemical facts, may well explain why the ferrous sulphate, when administered orally, is more toxic than the carbonate or the gluconate, since the liberation of the sulphuric acid into the surrounding tissues with the resulting cellular damage may well expose these tissues to the necrotising effect of the absorbed iron.

It also seems likely that, if this theory is correct, the necrotising effect of the liberated sulphuric acid breaks down the mucosal-ferritin block, which is held to control the absorption of iron from the gut, and so permits large amounts of iron to enter the general circulation, causing systemic toxicity. It is pertinent also to note that, whilst there is considerable variation between the toxicities of the iron salts when administered orally, they are all equally toxic when injected intravenously (D'Arcy and Howard, 1962*a*), presumably because under these conditions it is only the iron itself that is exerting a toxic effect.

Thus, in both acute and subacute toxicity tests, ferrous carbonate, in contrast to ferrous sulphate, has been shown to be of low toxicity. That it is also well absorbed has been demonstrated by its effect in raising the lowered haemoglobin levels in experimental iron-deficiency anaemia. In addition, the results of clinical trials (as yet unpublished) with the commercial preparation of ferrous carbonate used in these experiments, have shown that it effectively raises the haemoglobin levels in human cases of iron-deficiency anaemia.

This study shows, moreover, that ferrous carbonate, in a stable pharmaceutical preparation, is effective and far less toxic than ferrous sulphate in the treatment of iron-deficiency anaemia, and it would seem that its preferential use might help to prevent the increasing accidental mortality among children.

SUMMARY

In subacute toxicity tests in dogs given commercial preparations of ferrous salts by mouth, ferrous carbonate was considerably less toxic than ferrous sulphate and appreciably less toxic than ferrous gluconate. Histological examination of sections of the stomach and intestine of these dogs showed that the tissue damage was related to the presence of iron in the cells.

Commercial preparation of ferrous carbonate effectively raised the haemoglobin levels in iron-deficiency anaemia in piglets.

Since ferrous carbonate has such a low degree of toxicity and yet effectively raises haemoglobin levels, its preferential use in the treatment of iron-deficiency anaemias would help to prevent the increasing number of deaths of children who accidentally ingest large numbers of iron tablets.

We should like to thank Mr C. J. Airey for his expert advice and cooperation, and also Miss C. J. Spearing, Miss J. M. Charvill and Mr C. Wilkins for their invaluable technical assistance.

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THE RELATION OF IRON AND COPPER TO HEMOGLOBIN SYNTHESIS IN THE CHICK.*

By C. A. ELVEHJEM and E. B. HART.

With the Cooperation of A. R. Kemmerer.

(From the Department of Agricultural Chemistry, University of Wisconsin, Madison.)

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Shortly after we had demonstrated in this laboratory (1) that nutritional anemia could be induced in rabbits by a diet of cow's whole milk, and that this anemia could not be corrected by inorganic iron unless it was supplemented with a natural food material, or a preparation from this material, we attempted to use chicks as experimental animals for anemia studies. A survey of the literature at that time indicated that chickens had been used rarely for studies of this nature. The only work found was that of Coppela, as he is quoted by Stockman (2), who was able to reduce the hemoglobin in cocks to 33 per cent by feeding a diet containing no iron and to increase it, in 5 days, to 65 per cent by the addition of ferric lactate.

We were interested in using chicks because they are readily available laboratory animals and because we wished to obtain results with animals other than rabbits. Since that time rats have also been used in this laboratory (3) and it was the work with this animal which demonstrated the importance of copper as a supplement to iron for hemoglobin building (4).

In this paper we wish to present some of the fundamental facts observed during the work with the chicks, and to give the results which demonstrated that copper has the same favorable effect upon the hemoglobin synthesis in the chick as it has in the rat.

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

EXPERIMENTAL.

Day old chicks were brought into the laboratory and placed in pens that were properly warmed and fitted with wire screen bottoms to prevent consumption of any refuse. White Leghorns were used almost entirely in all the work. The first group was placed on a diet of cow's whole milk, but, although there was a noticeable decrease in the hemoglobin content of the blood, the chicks were unable to consume enough of the liquid milk to obtain sufficient nourishment. An attempt was made to eliminate this difficulty by supplementing the liquid milk with whole milk powder. This also was found to be unsuccessful because normal growth was not maintained. Therefore, it was decided that some food material which possessed considerable bulk must be added to the milk diet. Polished rice was chosen as the supplement because the iron content of rice is lower than that of any of the other cereals. The addition of cracked rice to the ration stimulated growth, but the anemia developed rather slowly. Finally it was found that if the rice was previously extracted with hot alcohol, this difficulty was eliminated. The rice was extracted in large percolators with alcohol at 37° for a period of 7 days. The alcohol was changed daily. The rice was then dried and 97 parts of the rice mixed with 2 parts of $CaCO_3$ and 1 part of NaCl. This mixture, together with cow's whole milk fed *ad libitum*, constituted the basal ration. The chicks were also irradiated for a period of 10 minutes every other day to insure the prevention of rickets.

When day old chicks were kept on this ration, they invariably developed anemia in 12 to 15 days. The amount of hemoglobin decreased from the normal of about 8 gm. per 100 cc. of blood to about 4 gm. per 100 cc. Samples of blood for hemoglobin determinations were obtained by puncturing one of the veins on the under-side of the wing. The hemoglobin was determined by the Dare hemoglobinometer in the earlier work and later the Newcomer method was used. When the Dare instrument was used, the figure for the gm. per 100 cc. of blood was calculated by multiplying the reading in percentage by the standard of 13.77 gm. given for this instrument. When the Newcomer method was used, the figure for the gm. per 100 cc. was obtained directly from the colorimeter reading.

It is not surprising to find this rapid drop in the hemoglobin content of the blood of chicks placed on a ration low in iron, because the reserve supply of iron in a chicken when hatched is very low. The iron content of an egg of average size is about 0.8 to 1.0 mg. of Fe. The amount present in the entire body of a chick at birth

TABLE I.

Hemoglobin Content of Chick Blood as Modified by Ferric Oxide and Lettuce Ash.

Chick No.	Diet.	Days on diet.			
		0	5	10	15
		Hb per 100 cc. blood.			
		gm.	gm.	gm.	gm.
202	Basal.	5.2	4.8	4.1	3.2
203		4.4	3.2	2.7	1.6
204		3.4	2.5	2.5	1.8
205		3.8	3.2	2.7	3.4
206		5.4	4.0	3.2	2.9
Average		4.4	3.5	3.0	2.6
208	Basal + Fe_2O_3 equal to 2 mg. Fe per chick per day.	2.7	3.2	3.3	4.5
209		4.8	4.5	4.5	4.8
210		1.4	2.3	2.1	2.3
211		4.5	3.4	3.7	3.8
212		3.7	4.1	3.8	3.2
Average		3.4	3.5	3.5	3.7
219	Basal + Fe_2O_3 equal to 2 mg. Fe per chick + lettuce ash equal to 1 gm. per chick per day.	3.8	8.0	8.0	9.6
220		4.1	8.0	7.7	9.6
221		2.7	5.5	6.6	8.0
222		4.3	7.3	8.9	8.3
223		5.1	6.2	6.5	10.3
Average		4.0	7.0	7.5	9.2

is between 0.6 and 0.7 mg. of Fe, most of which can be accounted for in the hemoglobin of the blood. This shows that the chick has practically no iron store from which additional hemoglobin can be built.

The chicks were allowed to become anemic by feeding them as a group in a large pen on the basal ration for a period of 12 to 15

days. The hemoglobin content of the blood of the individual chicks was then determined, and the chicks placed in small cages, five chicks in a group. Various additions were then made to each group of chicks.

Table I gives typical records of a group of five chicks continued on the basal ration 18 days after the preliminary feeding, a group

TABLE II.

Hemoglobin Content of Chick Blood as Modified by Soluble Iron Salts.

Chick No.	Diet.	Days on diet.		
		0	6	12
		Hb per 100 cc. blood.		
		gm.	gm.	gm.
246	Basal.	3.9	3.0	2.8
247		4.9	3.2	3.2
248		3.6	2.8	1.8
249		4.7	3.4	3.4
250		4.9	4.4	3.6
Average		4.4	3.4	3.0
287	Basal + $FeSO_4 \cdot 7H_2O$, Sample II, equal to 2 mg. Fe per chick per day.	3.6	7.9	8.3
288		4.8	7.9	8.4
289		3.8	6.6	8.5
290		3.8	6.2	7.5
291		4.8	6.6	7.9
Average		4.2	7.0	8.1
424	Basal + $FeSO_4 \cdot 7H_2O$, Sample IV, equal to 2 mg. Fe per chick per day.	4.9	6.9	6.3
425		4.8	4.8	7.2
426		4.8	6.9	9.2
427		3.7	7.6	8.3
428		3.7	7.4	8.8
Average		4.4	6.7	8.0

fed Fe_2O_3 with the basal ration, and a group fed lettuce ash in addition to the iron supplement. There is a continual deterioration of the blood stream in the chicks continued on the basal ration. When the chicks are fed ferric oxide alone, there is no increase in the hemoglobin content of the blood. As soon as lettuce ash is added, there is the same characteristic improvement as was noted previously in the work with rabbits (5).

Several groups of chicks were also given iron additions in the form of ferrous sulfate. Whenever this salt was fed, rapid and complete recoveries were made. The records of two groups fed ferrous sulfate are given in Table II. Ferrous sulfate Sample II was made from iron wire and ordinary sulfuric acid. Sample IV was made from iron wire and purified sulfuric acid. These records are typical of all the chicks that received a soluble iron salt. We attempted to prepare the salts in the purest possible form but the source of the iron in each case was iron wire, which might contain small amounts of impurity. Since these salts were all potent, we felt that the rapid response was probably due to the presence of some other element found in the iron salt as a contaminant.

Because ferric oxide did not bring about an improvement, we thought that this salt was free from the contaminating element and could therefore be used together with the basal ration in the determination of the element active in hemoglobin synthesis. A large number of salts of different elements were fed to the basal Fe_2O_3 ration. It is interesting to state that $CuCl_2$ was fed with Fe_2O_3 to chicks as early as April, 1926. No improvement in the blood stream was noted when any of these salts was fed together with Fe_2O_3.

The hemoglobin of all the chicks remained so uniformly low that the probability that this continued anemic condition was due to the lack of an available iron supply presented itself. Perhaps the iron in the ferric oxide was not being utilized by the growing chick. This assumption appeared quite possible since the feeding of a soluble iron salt resulted in an increase in the hemoglobin of the blood.

This fact suggested an experiment which would demonstrate definitely whether the iron in ferric oxide can be utilized by chicks. Three groups of fifteen chickens each were used for this work. One group was allowed to remain on the basal diet after preliminary feeding, one group was given Fe_2O_3 equivalent to 2 mg. of Fe per chick per day, and one group was fed $FeSO_4 \cdot 7H_2O$, Sample XI, at the same level of iron intake. This ferrous sulfate was prepared from electrolytic iron and sulfuric acid. The hemoglobin was followed for 10 days after which time the chicks were killed, the livers removed, dried, and analyzed for iron. The results are given in Table III.

The figures show conclusively that the iron content of the livers from the chicks receiving ferric oxide was no higher than the iron in livers of the chicks on the basal ration. The iron in the livers of the chicks fed the ferrous sulfate was over 6 times as high. The ferric oxide is therefore not assimilated by chicks. This fact explains the impotence of ferric oxide when used as a source of iron. This finding is also of practical importance since it shows that if iron is added to the ration of chicks it should not be in the form of ferric oxide.

The only alternative, therefore, was to use a soluble iron salt in further anemia studies with chicks. Since all the soluble iron salts used up to this time had stimulated hemoglobin regeneration, we turned our efforts to the purification of various iron salts to such an extent that they would not stimulate hemoglobin formation when fed alone. The preparation and purification of iron salts for chicks continued until it was demonstrated by the use of rats that pure iron salts were ineffective in hemoglobin synthesis unless the iron was accompanied by minute quantities of copper.

When the pure iron salts which had been found ineffective with rats and which had been found copper-free by actual test, were fed to chicks, a response similar to that noted with all soluble salts was obtained. The iron salt used in most of the work was $FeCl_3$, which was prepared in exactly the same manner as described in a previous publication (6).

TABLE III.

Effect of Fe_2O_3 and $FeSO_4$ on Hemoglobin Content of Blood and Iron Content of Liver of Chicks.

Group No.	Diet.	Days on diet. 0	5	10	Iron content of dry liver.
		Hb per 100 cc. blood.*			
		gm.	*gm.*	*gm.*	*per cent*
1	Basal.	4.1	3.8	3.7	0.0119
2	" + Fe_2O_3.†	4.0	4.1	4.2	0.0103
3	" + $FeSO_4 \cdot 7H_2O$†, Sample XI.	3.4	6.2	6.6	0.0672

* Figures for hemoglobin and iron content of liver are the averages of the fifteen chicks in each group.

† Iron salts fed at levels equivalent to 2 mg. of Fe per chick per day.

In Table IV we present in detail the results obtained by the addition of pure $FeCl_3$ to the basal diet and the addition of pure $FeCl_3$ supplemented with copper. It is readily seen that the pure $FeCl_3$ fed alone stimulated hemoglobin formation as well as the ferric chloride supplemented with copper. The stimulation in

TABLE IV.

Effect of a Pure Iron Salt and This Iron Salt Plus Copper on Hemoglobin Regeneration When Fed with Basal Ration.

Chick No.	Diet.	Days on diet. 0	6	12	18	24
		Hb per 100 cc. blood.				
		gm.	gm.	gm.	gm.	gm.
1250	Basal.	3.5	3.1	2.5	2.6	2.4
1251		3.8	3.1	2.6	2.1	Dead.
1252		3.8	3.3	3.2	3.8	"
1253		4.2	3.3	3.3	4.3	3.3
1255		4.0	3.2	2.9	4.2	3.1
Average		3.9	3.2	2.9	3.4	2.9
1256	Basal + 0.1 mg. Fe as $FeCl_3$	2.6	6.0	8.3	7.9	7.6
1257	(purified) per chick per day.	4.0	6.2	8.3	6.7	Dead.
1258		4.0	6.4	8.1	Dead.	
1259		3.9	4.4	6.4	6.7	6.7
1260		3.8	7.2	8.4	8.3	7.6
Average		3.6	6.0	7.9	7.4	7.3
1261	Basal + 0.1 mg. Fe as $FeCl_3$	3.8	6.6	6.9	7.9	7.2
1262	(purified) per chick per day,	3.3	7.0	8.7	8.9	7.8
1263	+ 0.01 mg. Cu as $CuSO_4$ per	4.0	7.3	8.6	8.5	8.6
1264	chick per day.	3.8	7.6	7.7	9.1	8.1
1265		2.7	6.5	7.6	7.4	7.3
Average		3.5	7.0	7.9	8.3	7.8

this case could not be due to any impurity in the iron salt, and we therefore turned our attention to the basal ration. It was thought improbable that the milk could furnish enough copper to effect hemoglobin synthesis because the work with rats had shown that when pure $FeCl_3$ was added to the milk ration of the rat, no regeneration took place. The only other source of copper supply

was from the rice preparation. Since the active element was known to be copper, we analyzed the rice preparation for this element and found it to contain 2.5 mg. of Cu per kilo. If a chick consumed 5 gm. of the rice per day, it would obtain 0.013 mg. of copper, which is a sufficient amount to stimulate hemoglobin

TABLE V.

Effect of a Pure Iron Salt and This Iron Salt Plus Copper on Hemoglobin Regeneration When Fed with a Modified Basal Ration.

Chick No.	Diet.	Days on diet. 0	6	12	18	24
		Hb per 100 cc. blood.				
		gm.	gm.	gm.	gm.	gm.
1362	Modified basal + 0.2 mg. Fe as	4.2	3.6	3.6	3.6	2.6
1363	$FeCl_3$ (purified) per chick per	4.3	3.7	2.8	3.3	Dead.
1364	day.	4.2	3.9	4.1	4.4	3.5
1365		4.0	3.6	4.0	4.7	4.9
1366		4.0	4.7	3.7	3.4	Dead.
Average		4.1	3.9	3.6	3.9	3.6
1368	Modified basal + 0.2 mg. Fe as	4.0	4.0	4.7	4.8	4.6
1369	$FeCl_3$ (purified) + 0.01 mg.	4.2	5.3	6.3	7.4	7.4
1370	Cu as $CuSO_4$ per chick per	4.0	6.6	6.2	6.6	7.2
1371	day.	3.9	5.8	6.4	7.7	7.0
1373		3.8	5.7	7.2	7.2	7.4
Average		4.0	5.5	6.2	6.7	6.7
1376	Modified basal + 0.2 mg. Fe as	4.0	5.6	6.2	6.5	Dead.
1377	$FeCl_3$ (purified) + 0.02 mg.	3.3	4.2	6.0	7.9	7.0
1378	as $CuSO_4$ per chick per day.	4.4	5.4	7.2	7.7	7.0
1379		4.8	6.5	6.1	6.4	5.3
1380		4.1	5.3	4.1	4.5	5.2
Average		4.1	5.4	5.9	6.6	6.1

synthesis. This fact explains immediately the results obtained with all the soluble iron salts. The basal ration was low enough in iron to produce anemia providing no soluble source of iron was supplied, but it was not low enough in copper to prevent hemoglobin building when the iron was supplied in available form.

In order to demonstrate conclusively that copper is the neces-

sary supplement to iron in the case of chicks as well as in rats, it was necessary to construct a basal ration which was practically free from copper. The difficulty of finding any food material other than milk which is exceedingly low in copper is readily seen by a study of the table giving the copper content of food materials which was published recently from this laboratory (7). The only material which looked at all promising was corn-starch. The starch was granulated by making a thick paste and drying at 37°. When this granulated starch replaced the rice in the basal ration, the chicks did not grow. The most decided deficiency of this ration seemed to be a lack of vitamin B because as soon as yeast was added good growth was obtained. Of course, yeast could not be used to supplement the ration since it contains considerable copper. A hot 90 per cent alcoholic extract of yeast also contained some copper. However, if this extract was evaporated to a thick paste, taken up in water, and extracted with ether, the largest portion of the copper was removed with the fat. In this way a fairly concentrated preparation of vitamin B, free from copper, was obtained. When this material, equivalent to 20 gm. of yeast, was added to 100 gm. of granulated starch, the ration so modified gave fair growth.

In Table V are given the results obtained when iron alone and iron and copper additions are made to this modified basal ration. When purified $FeCl_3$ is added to this ration, no improvement in the hemoglobin content of the blood takes place. As soon as 0.01 mg. or 0.02 mg. of copper as copper sulfate is fed each chick daily, a decided increase in the hemoglobin content is noted.

DISCUSSION.

The results presented in this paper verify again the great anemia-producing power, *i.e.*, the low iron and copper content, of whole milk. There seems to be no difference in the animal used; as soon as it is restricted to a milk diet directly after weaning, anemia develops rapidly. However, in the case of an animal like the chick, which will not grow normally on a milk diet alone, the introduction of additional food, unless highly purified, has a favorable effect upon hemoglobin synthesis. These results, as well as actual analyses of these foods for iron and copper, demonstrate the universal distribution of these hemoglobin-building elements in

most natural foods. This fact is of great importance whether one is interested in producing anemia in experimental animals on any diet other than milk, or whether one is interested in the use of natural foods as a supplement to milk for the prevention of anemia in young growing animals.

One must be exceedingly careful in the preparation of food materials other than milk for the production of anemia in experimental animals. Several natural foods may be purified to such an extent that they are low enough in iron to produce anemia, but it is very difficult to reduce the copper content enough to maintain the anemia when sufficient iron is added to the diet. The modified basal ration used in the chick work presented was low enough in copper to maintain anemia when it was supplemented with pure ferric chloride, but the growth of the chick was not entirely normal.

Recently Drabkin and Waggoner (8) reported that the severe anemia developed in rats on a milk diet could be cured by placing these rats on a copper-free synthetic ration. These workers were so kind as to furnish us a sample of their copper-free synthetic ration for analysis. Our analyses show that this ration contained 0.044 mg. of copper and 0.532 mg. of iron per 10 gm. of the ration. Assuming the rats consumed 10 gm. each of the ration per day, they would be ingesting the optimum amounts of iron and copper for hemoglobin regeneration. It certainly would be surprising if a ration compounded of materials such as egg albumin, commercial casein, and dry brewers' yeast did not contain some copper. The importance of careful estimations of copper in anemia-producing diets cannot be overemphasized.

CONCLUSIONS.

Day old chicks placed on a diet of cow's milk together with polished rice, calcium carbonate, and sodium chloride, invariably become anemic. The hemoglobin falls from 8 gm. per 100 cc. of blood to 4 gm. per 100 cc. in 12 to 15 days.

Additions of ferric oxide to this ration will not prevent the anemia because the iron in ferric oxide is not assimilated by chicks.

The addition of ferrous sulfate or purified (copper-free) ferric

chloride to this ration immediately stimulates hemoglobin synthesis because this basal ration contains small amounts of copper.

When purified ferric chloride is added to a modified basal ration very low in copper, no stimulation is noted until minute amounts of copper are added.

Copper acts as a supplement to iron in hemoglobin synthesis in chicks as well as in rats.

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Acute Ferrous Sulfate Poisoning in Children; Report of Five Cases

GEORGE C. EMMANOUILIDES, M.D.*

The medical profession is becoming more and more aware of the fact that iron preparations are not inocuous medications. They may produce severe, and in many instances fatal, poisoning when accidentally ingested by the inquisitive toddler.

* Research Fellow in Hematology, Children's Hospital.

Although iron preparations have been present for many centuries in the medical armamentarium it is strange that the report of iron toxicity which initiated the current interest appeared as late as 1947, when Forbes[1] reported two fatal cases from England. Since then quite a number of reports have appeared in the English and American literature,[2-24] thus supporting the fact that iron poisoning is not an uncommon occurrence in late infancy and early childhood. The importance and the seriousness of this condition have been emphasized, as well as the recommendation that iron preparations should no longer be considered nontoxic, and several suggestions regarding the pathogenesis and treatment have been made.

Acute ferrous sulfate poisoning has been observed in five instances within a period of less than six months at Children's Hospital. One patient died, one was severely affected, and three were mildly affected. Clinical and laboratory observations with a brief discussion are presented in this report.

CASE REPORTS

Case 1.

B. C., an 18 month old Negro girl, was brought to the outpatient department at 5:00 P.M., September 19, 1958 approximately two hours after ingesting 20 to 25 tablets of ferrous sulfate (3 grains each). One hour after ingestion the child vomited a brownish fluid which contained a few red strands of blood. The mother noticed a few scattered "pills" on the floor and immediately brought the child to the hospital. The child was alert on arrival and gastric lavage was performed. Toward the end of the procedure she became lethargic for the first time.

Physical examination at that time revealed a well developed and well nourished semicomatose child. She responded only sluggishly to painful stimuli. Her respirations were deep and regular. Her pulse was 130 per minute and her blood pressure 90/50. Her eyes were moderately sunken and the pupils reacted sluggishly to light. The corneal reflexes were absent and there was slight cyanosis of the lips. The deep tendon reflexes were decreased. During the initial examination she twice vomited a brown watery vomitus which contained a small amount of bloody mucoid substance. Laboratory data are summarized in table 1.

She remained in deep coma until about 9:00 P.M. when for the first time she cried during a venous puncture. Meanwhile her blood pressure dropped to 70/30 and 100 ml. of whole blood was administered and was followed by continuous intravenous infusion of fluids. Fifty milligrams of vitamin K (Mephyton®) was given intravenously. Her pulse and blood pressure remained stable, but she continued to be lethargic. At midnight she was more responsive and rather irritable. Two loose greenish black stools were reported. The following morning she was more alert and breathing normally. Moderate acidosis developed subsequently and was corrected with intravenous fluids. She was again given 125 ml. of whole blood because of a falling hemoglobin (from 10.0 to 8.8 Gm. per 100 ml.). On the second hospital day her condition improved remarkably and she was discharged eight days later in satisfactory condition. On March 19 an x-ray examination of the upper gastrointestinal tract showed no evidence of obstruction.

Case 2.

D. W., a 3 year old white girl, was brought to the hospital October 8, 1958 with a history of having eaten "iron pills" of unknown quantity approximately one hour

TABLE 1

Laboratory Data of Case 1

Date and Time	9-19-58 6:30 P.M.	9:30 P.M.	12:00 P.M.	9-20-58 9:00 A.M.	3:00 P.M.	9-21	9-22	9-23	9-26	9-27
Serum iron (mcgm/100 ml)	3194	2000	1277	736	513		222		125	
Prothrombin activity (% of normal)		27.0			45.0	56.0			23	91
Leukocyte count		32,200			16,400			9,100		
Sodium (mEq/L)		138.0			120.0	135.0				
Potassium (mEq/L)		3.5			3.4	4.5				
Chlorides (mEq/L)		111.0			94.0	106.0				
CO_2 (mEq/L)		14.1			10.0	16.8				
Total bilirubin (mg/100 ml)							2			1
Cephalin flocculation							1+			1+

previously. Following the ingestion she had vomited a greenish brown watery material three times. No diarrhea was noted. On physical examination she was found to be well developed and well nourished and rather pale. Her respirations were normal. Her pulse was 140 per minute and her blood pressure 125 75. Yellowish green discoloration of the teeth was noted. The remainder of the examination was not remarkable. Gastric lavage was performed but no evidence of ingested tablets was found. During the two hours following admission she became slightly lethargic and her blood pressure fell to 80 60. She was treated with intravenous fluids. The only abnormal laboratory finding was an elevated serum iron, 597 mcgm. per 100 ml., two and one half hours after the ingestion of the tablets. She was discharged two days later in satisfactory condition.

Case 3.

C. H., a 2 year old Negro girl, was brought to the emergency room November 24, 1958 with a history of swallowing an unknown quantity of "iron pills" two hours earlier. The tablets had been given to her post partum mother a few days previously. While at home the patient had vomited four times, but no diarrhea had been noted. Upon arrival she was found to be alert and rather irritable. Her pulse was 135 per minute and her respirations were normal. Her blood pressure was 100 68. The remainder of the physical examination was not remarkable. Gastric lavage was performed with 400 cc. of mild bicarbonate solution. The only abnormal laboratory finding was an elevated serum iron of 639 mcgm. per 100 ml. which the following day was reported as being 472 mcgm. per 100 ml. She had an uneventful hospital course and was discharged two days after admission.

Case 4.

W. T., an 18 month old Negro girl, was in good health until midnight of December 22, 1958 when she developed persistent vomiting and watery diarrhea. Approximately 18 to 20 tablets were seen in her vomitus. Her mother recognized them as some given to her a few weeks previously at the time of a post partum check up. The exact number of ingested tablets was not known. The vomiting and diarrhea continued for about two hours. The patient subsequently became comatose and remained in this state for about six hours. The mother thought that the child was "sleeping." About 30 minutes prior to arrival at the hospital the patient awoke and began

to cry. This was soon followed by coma, and the patient was rushed to the hospital. Upon arrival she was found to be in a deep coma with slow, superficial and irregular respirations. The pulse was very weak and irregular, and no blood pressure could be detected. She was pronounced dead a few minutes later before treatment could be instituted. Autopsy results are not available.

Case 5.

On February 17, 1959 C. E., a 3 year old Negro boy, swallowed an unknown number of ferrous sulfate tablets and subsequently developed vomiting. The vomitus contained chewed-up tablets with white particles in it. He became very thirsty and refused his supper. Three hours later he vomited again and had a loose watery stool. He was brought to the hospital and a gastric lavage was perfomed. On physical examination he was alert and irritable. His respirations, pulse and blood pressure were normal. His tongue was brownish in color. A few impetiginous lesions were present over both extremities. The neurological examination was not remarkable. His serum iron level on admission was 766 mcgm. per 100 ml. The other laboratory examinations performed were within normal limits. His hospital course was uneventful. He was treated with intravenous fluids and was discharged after two days in good condition.

DISCUSSION

In a review of 42 case reports of iron poisoning from the medical literature, Aldrich,[2] in 1957, noted that approximately 50 per cent of the reported cases were fatal. It is apparent that many more instances of acute iron poisoning occur, since mild cases usually are not being reported and other moderately severe cases are overlooked. The fact that our cases have occurred during a period of less than six months supports the above statement. In all our cases the iron was taken accidentally in the form of 3-grain ferrous sulfate tablets. This medication had been given to adults for treatment of anemia (in four instances to the mother, prenatally or postnatally). Cases 2, 3 and 5 were mild, the main symptom being vomiting. Diarrhea and lethargy of short duration were noted in one case. All 3 of these patients had an uneventful recovery and were discharged on the third hospital day. The only abnormal laboratory finding in these 3 children was an elevation of the serum iron which ranged between 597 and 766 mcgm. per 100 ml. (the normal serum level of iron is 125 to 175 mcgm. per 100 ml.). All blood samples were obtained approximately two and one-half to three and one-half hours after ingestion of the tablets. We believe that if proper facilities are available, a serum iron determination is the best laboratory test for confirming the diagnosis and detecting the severity of the poisoning.

Case 1 represents a rather severe case of iron poisoning, and resembles in its clinical picture most of the previously reported cases.

Acute iron poisoning can be divided clinically into four chronological phases.[2, 3, 4] The first phase is characterized by vomiting, diarrhea and, subsequently, symptoms of cardiovascular collapse, lethargy and coma. It lasts approximately six to eight hours. One fifth of the fatal cases occur

in this phase. The second phase is characterized by a temporary improvement of the patient and lasts 10 to 16 hours. This phase is very important because at any time a sudden relapse of a severe and usually irreversible cardiovascular shock may occur. The latter represents the third phase and may occur 20 to 56 hours after ingestion of the poison. Seventy-five per cent of the fatal cases usually succumb during this phase. The last phase occurs as a very late manifestation of this clinical syndrome in those patients who recover from the more severe form. Signs and symptoms of gastrointestinal obstruction, secondary to scarring, may appear one month or more later. Surgical intervention is necessary when this complication occurs.[16, 19, 23]

The age incidence of our cases was between 18 and 36 months, similar to those observed in other reports.[2, 10, 21] All cases were from the lowest socio-economic class, and there were four Negroes and one white. It was not possible to determine the exact dosage of the ingested tablets except in case 1. The fatal dosage varies widely. Spencer[10] reported fatal poisoning with 1.8 Gm. of ferrous sulfate, while recovery has followed ingestion of 15 Gm.[10, 11] It is apparent that factors such as fullness or emptiness of the stomach before the ingestion, or vomiting which occurs soon after, may bear a relationship to the severity of the poisoning.

Pathologic findings include necrotizing gastritis, and congestion and ulceration of the mucosa of the stomach or small intestine.[5, 17, 21, 22] Mesenteric lymphadenitis and thrombosis of the mesenteric veins may occur.[13] Cloudy swelling and periportal hemorrhagic necrosis of the liver and other viscera have been observed.[17]

In individuals suffering from acute iron poisoning, very high plasma iron levels have been demonstrated together with a marked leukocytosis, increased serum bilirubin and decreased carbon dioxide combining power. Decreased prothrombin activity was observed in case 1 and a similar finding has been observed by others.[5] There have been no reports of blood coagulation abnormalities among the survivors.[5]

There is a suggestion that some correlation between the serum iron level and the severity of the poisoning exists. Serial examination of serum iron in case 1 showed results similar to those observed by others (see tables 2 and 3). In 7 cases of iron intoxication reported in the literature serum iron determinations were performed. A level as high as 8,150 mcgm. per 100 ml. of serum iron has been observed in a patient who survived.[5] The lowest reported iron level was 1,680 mcgm. per 100 ml.[24] In this case drowsiness and lethargy were observed. There have been no reports of serum iron levels in fatal cases. In case 1 the highest level observed was 3,194 mcgm. per 100 ml.; three hours later this had dropped to 2,000 mcgm. per 100 ml. At this time, when the blood sample was taken, the child began to respond

TABLE 2

Serum Iron Levels Correlated with Clinical Findings as Recorded from the Literature

Author	Amount Ingested	Initial Serum Iron	Coma	Drowsiness	Outcome	Serum Iron after 24 Hours
	Gm.	*mcgm/100 ml*				*mcgm/100 ml*
Spencer[10]	15	3,300	+		Recovered	1,120
	6	3,420	+		Recovered	330
Kaplan et al.[24]	6	1,680		+	Recovered	normal
Birk et al.[7]	13	4,000	+		Recovered	2,000
Brown and Gray[21]	8	5,450	+		Recovered	450 (42 hours)
Davis and Gibbs[5]	?	8,120	+		Recovered	
Amerman et al.[9]	4	3,500	+		Recovered	

TABLE 3

Serum Iron Levels Correlated with Clinical Findings in Four Cases of Iron Intoxication Seen at Children's Hospital

Case No.	Age	Initial Serum Iron	Serum Iron after 24 Hours	Vomiting	Diarrhea	Drowsiness	Coma
	months	*mcgm. 100 ml*					
1. (B. C.)	18	3,194	513	+	+		+
2. (D. W.)	36	597	139	+	+	+	
3. (C. H.)	24	639	472	+			
5. (C. E.)	36	766		+			

to stimuli and cried. Subsequent determinations showed a steady decline, while the general condition of the patient steadily improved. On the third hospital day the serum iron was within normal limits. From the above observations there is some evidence that high serum iron levels are associated with coma during the first phase. There appears to be a wide variation in the serum iron levels associated with central nervous system depression. The latter usually is seen with iron levels over 2,000 mcgm. per 100 ml.

The exact mechanism of death during the first phase is not known. Large amounts of ferritin are released from the gastrointestinal tract into the blood due to the local effects of iron.[15, 22, 25] As ferritin is a potent vasodepressor, absorption of large amounts could explain the shock observed in this phase.[25] Brown and Gray[21] postulated that a direct toxic action of uncombined iron also exists during this phase. The mechanism of death during the third phase is attributed to severe liver damage.

There is no specific treatment for this poisoning. General supportive measures have been recommended. Emptying the stomach by induction of

vomiting or gastric lavage and the use of whole blood, plasma or plasma substitutes as supportive measures to overcome the shock are the main steps in treatment. Correction of the electrolyte imbalance with proper intravenous fluids is necessary. Our case 1 was treated only by supportive measures and the child had an uneventful recovery. The use of BAL is controversial.[31] The use of the artificial kidney has been suggested but it is important to consider the fact that a large part of the iron in the serum remains in the ferric state and cannot be dialyzed.[29, 30] Chelating agents have been used but no published reports are available as yet.[21] Exchange blood transfusion was recently performed in one case with apparently good results.[9] We believe that exchange transfusion carefully performed within the first six hours after the ingestion of the iron may greatly benefit the severely poisoned child.

As in any accidental poisoning of early childhood, the most important responsibility for prevention lies with the parents. They should be warned that "iron pills" are dangerous for children and should be carefully locked up in an inaccessible place. The physician and the pharmacist should realize the hazard of prescribing large numbers of tablets to pregnant women or to blood-bank donors. In contrast, small numbers of tablets packaged in tight wrappings should be delivered to the iron-deficient adult. In four of our cases an uncounted number of "iron tablets" were given to the mother by an obstetrical outpatient clinic.

SUMMARY

Five cases of acute accidental ferrous sulfate poisoning are reported. Three of them were mild, one severe, and one fatal. The incidence of this poisoning is not uncommon. Serial determinations of the serum iron in the severe case were performed, and the observations made are recorded. The clinical picture, pathological findings, pathogenesis, treatment and prevention of this syndrome, with a brief review of the English literature, are discussed. We believe that treatment with exchange transfusion during the first four to six hours may greatly benefit the severely poisoned child.

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Iron Supplementation During First Year of Life

JOHN D. FARQUHAR, MD
PHILADELPHIA

Introduction

It is now a well-established fact that the so-called physiologic anemia of late infancy[1] —occurring between 6 and 18 months of age —is an iron deficiency anemia. It is characterized by decreased hemoglobin concentration, decreased hematocrit, microcytosis, and hypochromia. This is in contrast to the "physiologic anemia of early infancy" which is due to a relative hypoplasia of the erythroid elements of the bone marrow and occurs in the presence of adequate iron stores.

The fact that iron supplementation—either orally from dietary or medicinal sources or from parenteral administration—will correct or largely prevent this iron deficiency anemia both in full-term and in premature infants is well accepted.[2,3] It has been generally assumed, however, that in "well-nourished" infants who are receiving an "adequate" source of dietary iron, the additional supplementation with medicinal iron would produce no significant difference in hemoglobin concentration or hematocrit values. The purpose of this study was to test this assumption.

This seemed to be one instance where a pediatrician in private practice was in a better position to obtain these answers than investigators working with a large clinic population. This study was conducted among infants from an above average intellectual and socioeconomic group. There was no reason to doubt that these infants received excellent parental care and an optimal diet or that the parents followed the instructions given them during the course of the study.

Thus the object of this study was to determine whether daily supplement of 5 mg of elemental iron given orally as ferric pyrophosphate would maintain hemoglobin and hematocrit levels at a significantly higher level during the first year of life.

Methods

All healthy full-term infants who entered my pediatric practice were included in this study. No other criteria were used for patient selection. Eight of the total of 52 subjects failed to complete the

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John D. Farquhar, MD, Lankenau Medical Bldg, Lancaster & City Line Avenues, Philadelphia 51, Pa.

one-year study—four because their mothers stopped the medication for some irrelevant reason and not because of adverse effects and four because their families moved away and could not be followed.

Using the double blind technique, a patient assignment sheet randomly assigned each infant one of four colors, two of which represented a multivitamin preparation and two the same multivitamin preparation to which iron as ferric pyrophosphate had been added.

The multivitamin preparation which was given once daily contained the following ingredients in each 0.6 cc dose: vitamin A, 5,000 units; vitamin D, 500 units; thiamine (vitamin B_1), 1.5 mg; vitamin B_6, 10 mg; pyridoxine (vitamin B_6), 1.0 mg; cyanocobalamin (vitamin B_{12}), 3 μg; riboflavin, 1.2 mg.

The preparations containing the iron included 43 mg of ferric pyrophosphate, which is equivalent to 5 mg of elemental iron in each 0.6 cc dose.

As can be seen, this vitamin preparation does not contain any vitamin C, because ascorbic acid is not stable in the presence of iron. In order to insure an adequate vitamin C intake, all of the infants were given separately 50 mg of ascorbic acid daily up until the time—at about two months—when they were drinking at least one ounce of orange juice daily.

The dietary regimen which these infants followed was as follows:

1. Breast milk or evaporated milk formula for the first four months, then homogenized milk.
2. Cereal was added at about one month.
3. Fruits were started at about six weeks.
4. Orange juice started at about two months.
5. Vegetables started at about two months.
6. Egg yolk was added at about three months.
7. Meats were started at about four months.

The solid food was subdivided into three meals as follows: AM: juice, cereal, egg yolk; NOON: meat, vegetable, fruit; PM: cereal, fruit.

In addition, these infants ingested between 29 and 30 ounces of milk daily.

No vitamin or iron supplement was given until one month of age when the infants entered this study. Hemoglobin and hematocrit determinations and weight and height measurements were made just prior to therapy at one month of age and were

TABLE 1.—*Multivitamin*

Sex	Pretherapy, 1 Mo				Approx. 3 Mo			
	Hgb (Gm)	Hct (%)	Wt (Lb)	Ht (In.)	Hgb (Gm)	Hct (%)	Wt (Lb)	Ht (In)
M	15.9	45	9.06	21.50	10.6	32	13.13	24.00
M	14.2	41	8.56	20.75	14.7	43	13.31	23.75
M	10.0	32	6.63	19.00	11.2	33	10.94	22.00
M	11.5	33	10.69	21.75	12.4	37	14.38	24.00
M	13.5	38	10.25	21.50	15.2	46	15.50	25.25
M	14.1	44	9.88	23.00	14.1	44	9.88	23.00
M	11.0	33	9.00	20.75	11.6	35	16.81	25.25
M	15.2	42	9.43	22.00	12.0	36	16.13	26.50
M	11.4	33	11.25	22.75	12.1	37	16.50	25.75
M	13.1	42	9.88	21.00	15.4	46	14.50	24.25
M	15.7	47	9.81	20.50	11.4	34	13.06	24.25
M	13.6	39	9.75	21.34	11.8	36	16.81	25.75
M	14.1	41	11.56	22.25	10.2	30	16.75	25.00
M	12.4	35	10.31	21.75	13.4	40	15.56	25.50
F	12.4	38	7.94	20.75	11.6	32	12.31	23.50
F	13.1	40	9.88	22.00	13.1	38	14.94	24.50
F	12.5	37	12.44	23.75	12.8	37	15.44	25.00
F	11.2	35	10.13	21.00	10.7	32	14.44	23.25
F	13.9	40	8.75	21.25	10.4	33	12.06	24.50
F	15.4	46	7.38	19.50	14.1	43	11.47	22.12
F	16.0	49	8.81	21.50	13.4	40	14.06	26.00
F	11.5	32	11.00	22.00	13.0	37	15.81	24.25
F	12.2	35	13.81	23.50	11.2	32	17.50	25.75
F	11.1	33	13.25	23.00	11.9	36	15.00	25.00
F	12.6	37	10.38	22.00	11.0	33	14.94	24.75
F	10.4	31	8.06	20.50	12.4	36	11.00	23.00
F	13.6	41	10.19	22.50	11.7	35	12.75	24.75
Averages								
(1)	13.0	38	9.93	21.60	12.4	37	14.33	24.47
(2)	13.1	39	9.92	21.55	12.4	37	14.10	24.25
(3)	13.1	39	9.62	21.25	12.4	37	14.09	24.25

Averages: (1) arithmetic average; (2) adjusted average (to common pretest values); (3) adjusted average for 44 who completed study

Sex	Pretherapy, 1 Mo				Approx. 3 Mo			
	Hgb (Gm)	Hct (%)	Wt (Lb)	Ht (In.)	Hgb (Gm)	Hct (%)	Wt (Lb)	Ht (In)
M	15.9	48	9.63	21.00	14.3	41	13.00	24.25
M	12.1	35	9.56	21.25	12.2	37	13.88	23.75
M	12.5	31	8.19	20.50	9.0	28	11.00	24.25
M	14.0	41	9.63	21.00	11.6	34	14.13	24.00
M	12.4	35	9.38	21.25	9.9	28	13.63	23.50
M	15.1	46	10.06	21.50	12.2	37	15.50	24.50
M	16.0	48	7.69	20.25	11.9	33	13.13	23.00
M	17.2	49	9.38	22.00	11.5	37	17.00	26.50
M	14.8	44	7.94	20.00	11.6	35	14.00	23.50
M	13.4	39	9.56	21.00	10.8	33	13.63	25.50
M	13.8	39	10.38	21.50	12.0	34	16.25	24.00
M	14.3	41	8.69	20.50	11.2	33	12.81	22.00
M	14.7	44	9.25	21.25	11.3	33	14.00	24.00
M	15.9	48	10.00	21.25	10.5	30	14.44	24.00
F	14.2	37	9.63	20.25	11.4	34	14.25	23.25
F	10.3	29	8.63	20.00	11.0	29	14.00	23.25
F	12.0	34	8.38	20.25	9.9	28	15.31	24.00
F	11.9	36	8.13	21.00	12.2	34	11.69	23.10
F	10.8	32	12.75	22.00	14.6	41	16.06	24.50
F	14.3	40	9.20	20.25	11.5	35	14.19	24.50
F	12.0	36	8.13	20.00	11.7	36	14.56	24.00
F	10.0	33	11.50	23.00	12.9	37	15.88	25.00
F	13.4	40	9.00	20.50	11.1	35	14.31	24.50
F	14.2	42	8.56	20.25	9.4	33	12.06	22.75
F	12.5	35	9.25	21.00	12.9	38	12.63	24.25
Averages								
(1)	13.5	39	9.31	20.91	11.6	34	14.18	24.01
(2)	13.1	39	9.62	21.25	11.5	34	14.41	24.23
(3)	13.1	39	9.62	21.25	11.7	34	14.61	24.30

Averages) (1) arithmetic average; (2) adjusted average (to common pre-test values); (3) adjusted average for 44 who completed study.

The differences in hemoglobin and hematocrit values between the two groups are significant at the 5% level at three, six, and nine months of age, but the difference does not attain statistical significance at 12 months. These hemoglobin and hematocrit values—after adjusting to a common pre-test value—are shown graphically in Fig 1 and 2, respectively.

A comparison of the weight and height measurements revealed no differences between the infants who received the iron supplement and those who did not. Comparing the males and females by adjusting all pre-test measurements to a common base line revealed no differences in hemoglobin, hematocrit, weight, or height between the sexes at any of the post-treatment intervals.

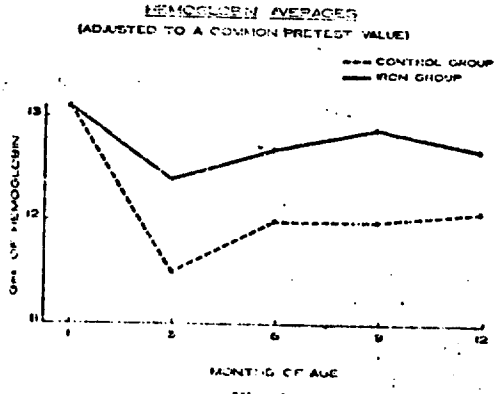

Fig 1

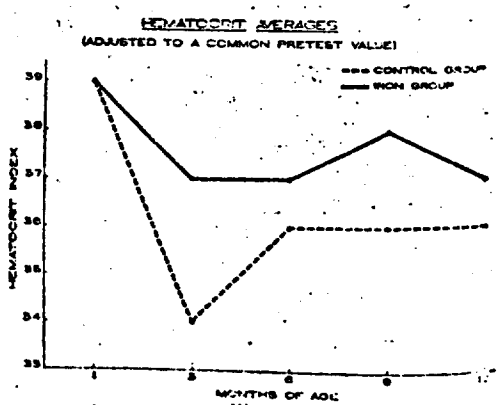

Fig 2

Control Group

Approx. 6 Mo				Approx. 9 Mo				Approx. 12 Mo			
Hgb (Gm)	Hct (%)	Wt (Lb)	Ht (In)	Hgb (Gm)	Hct (%)	Wt (Lb)	Ht (In)	Hgb (Gm)	Hct (%)	Wt (Lb)	Ht (In)
13.2	36	16.63	27.00	12.6	39	19.63	29.00	13.0	39	21.31	31.00
12.5	35	17.81	26.50	12.0	37	20.06	28.50	12.4	37	23.39	29.75
9.5	29	19.00	28.50	11.0	33	23.25	30.25	11.5	33	25.06	31.25
12.5	37	17.69	26.50	12.55	37	20.31	27.25	12.6	37	23.00	30.50
12.3	39	16.39	26.50	11.4	35	21.69	28.75	13.5	38	24.06	29.75
13.4	38	15.70	28.00	13.35	37.5	20.03	29.50	13.3	37	21.56	31.00
12.3	39	15.44	25.75	11.7	37	19.50	28.50	11.0	34	22.25	30.25
12.4	37	21.31	28.75	12.3	39	24.63	30.75	12.3	36	25.88	32.50
10.8	32	15.13	26.50	10.0	30	20.50	27.50	9.3	31	20.63	28.25
12.6	39	16.75	28.00	12.8	39	17.94	28.75	13.0	39	19.13	29.75
11.7	35	15.76	27.00	12.15	35.5	20.31	27.50	12.6	36	22.13	28.00
12.5	37	15.19	25.25	12.5	37	19.75	27.00				
12.25	35.5	16.53	26.12	13.2	38	19.06	28.25				
11.6	34	16.75	24.75								
12.2	35	16.81	25.00	12.1	37	17.21	26.50	12.1	37	18.88	26.00
10.5	33	19.50	27.50	11.35	35	22.16	29.12	9.1	29	24.81	30.75
11.3	36	20.44	25.25	10.7	31	23.81	29.00	10.3	31	26.06	30.50
12.5	40	14.13	26.00	12.4	38	17.00	28.75	14.5	44	18.63	29.75
11.8	36	18.56	25.50	12.9	40	22.63	26.50	14.1	41	23.75	29.50
11.6	35	19.06	27.00	13.1	38	21.75	29.00	12.3	35	23.50	30.00
11.0	33	20.06	28.75	10.4	33	23.56	30.50	10.3	30	25.31	32.25
12.2	37	20.31	27.00	12.5	37	24.63	29.00	12.5	36	26.75	32.00
12.0	36	18.31	27.50	10.7	33	21.00	28.50	11.4	32	22.38	32.50
12.1	36	14.44	24.50								
13.9	40	16.35	26.00								
12.0	36	17.72	26.72	12.0	36	20.59	28.65	12.1	36	22.93	30.54
12.0	36	17.86	26.84	12.0	36	21.03	28.78	12.1	36	23.02	30.49
11.9	36	18.30	27.06	11.9	36	21.16	28.88	12.1	36	23.02	30.49

Thus, the results show that the administration of 5 mg of elemental iron daily from one month to one year will produce a statistically significant increase in hemoglobin and hematocrit values at three, six, and nine months of age. This difference is not statistically significant at 12 months of age. The increase in hemoglobin and hematocrit values in the iron supplement group does not reflect itself in weight and height measurements.

Comment

The results of this study indicate that hemoglobin and hematocrit values can be increased significantly by iron supplementation during the first year of life with as little as 5 mg of elemental iron daily, given orally to a group of very well-nourished, healthy infants seen in private practice.

Sturgeon[3] concludes "that a daily dietary allowance of 1.0-1.5 mg/kg/day will achieve optimal iron nutrition for a substantial majority of the infant population." This investigator estimates, in his group of infants from a high socioeconomic group, that at three months of age they received 4-5 mg of dietary iron per day and at six months 8 mg of iron per day, or about 1 mg/kg/day. The addition of 5 mg of medicinal iron per day will increase the intake to about 1.5 mg/kg/day. The results of my present study indicate that this iron supplement did produce a statistically significant difference and that the higher figure of 1.5 mg/kg/day is necessary to provide optimal iron nutrition. Sturgeon has found that hemoglobin concentrations were not increased further by giving orally more than 1.5 mg of iron/kg/day.

In Sturgeon's well-nourished group of infants ("superior normal control group") the mean hemoglobin values were 11.3 gm and 11.6 gm at 6 and 12 months, respectively, with no significant difference from a group of infants who received 1.0 and 2.0 mg/kg/day of iron as ferrous sulfate. The corresponding values in my study were 12.0 gm and

12.1 gm at 6 and 12 months, respectively, in the control group as compared to 12.7 gm and 12.7 gm in the infants who received 5 mg per day of iron as ferric pyrophosphate. The difference of 0.7 gm of hemoglobin is statistically significant. It is interesting that this difference equals the increase of 0.7 gm which Sturgeon observed when he gave his "superior normal control group" 250 mg of parenteral iron.

Summary

The purpose of this study was to determine whether the daily ingestion of an iron supplement (5 mg of elemental iron as ferric pyrophosphate) would elevate significantly hemoglobin and hematocrit levels or increase the rate of growth during the first year of life in a group of healthy, well-nourished infants. Hemoglobin and hematocrit levels and weight and height measurements were taken at 1, 3, 6, 9, and 12 months. Starting at one month of age, half of the group in this double-blind study received daily 5 mg of iron in a multivitamin preparation and the control group received the multivitamin alone. The iron supplement produced a statistically significant increase in hemoglobin and hematocrit levels at three, six, and nine months of age, but the difference was not statistically significant at one year. This difference did not reflect in the height and weight measurements or in the general well-being of these well-nourished, healthy infants. Comparing the males and females at each post-treatment interval, there was no difference in hemoglobin, hematocrit, weight, or height between the sexes.

In conclusion, although a significant statistical difference was shown in the hemoglobin and hematocrit values by giving the iron supplement, there is no evidence that this difference has any real medical significance.

S. M. Free, PhD, bio-statistician, analyzed the above data for statistical significance.

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POISONING WITH A PREPARATION OF IRON, COPPER, AND MANGANESE

BY

GILBERT FORBES, M.D., B.Sc., F.R.F.P.S., F.R.C.S.Ed.

Lecturer in Forensic Medicine at the University of Sheffield; Police Surgeon to the City of Sheffield

Most iron salts are relatively inert, and modern therapeutic practice recommends their use in full doses. Certain iron preparations are, however, apt to cause dyspepsia, and even one Blaud's pill may occasion abdominal discomfort in susceptible individuals. According to Goodman and Gilman (1943) iron salts used in the treatment of anaemias may cause "gastric distress, colicky pain, and diarrhoea. These complaints are more prominent after ferric salts than after ferrous salts, and more common with the soluble than the insoluble preparations." They consider that this is especially true of ferrous sulphate, owing, in part at least, to the smaller doses used.

Cases of poisoning due to the ingestion of iron are extremely rare. Smith and Cook (1934) mention a case of a girl who swallowed 1 oz. (28 g.) of ferrous sulphate and recovered. Nearly all the cases of poisoning by iron preparations are due to the tincture of perchloride of iron, but a case of iron encephalopathy has been reported by Hurst (1931) following the oral administration of huge doses of iron and ammonium citrate. He states that no other example of remote symptoms due to iron (other than local gastro-intestinal effects) has been reported.

Some experimental work has been done on iron poisoning, but for the most part the method of administration has been by injection. McGuigan (1926) quotes Kunkel to the effect that the fatal intravenous dose of iron for dogs is from 20 to 50 mg. per kg. of body weight. Meyer and Williams, according to McGuigan (1926), found that 0.6 g. of ferrous sulphate injected into the veins of a dog caused pronounced vomiting and diarrhoea; 8 g. given orally proved fatal to a dog in 26 hours, and the necropsy showed ecchymosis of the stomach and intestines. McGuigan also reports the death of a man following the ingestion of 45 ml. of tincture of iron.

Copper sulphate falls into the category of irritant metallic poisons. Acute poisoning with this substance is very rare, and fatal cases are still more uncommon. Consequently the fatal dose of this salt is unknown, but Smith and Cook (1934) advance the suggestion that doses of 1/2 oz. (14 g.) and upwards would act as powerful irritants on adults, and that a much smaller quantity would suffice to destroy infants or children. Copper sulphate is a powerful emetic, and may be used clinically for this purpose in doses of from 5 to 10 gr. (0.32 to 0.65 g.). If taken in larger quantities it causes acute gastro-enteritis. Because of its irritant properties, if this salt is given as an emetic and fails to act the stomach must be promptly emptied by some other means (Douthwaite, 1931). Retained copper is absorbed from the intestine and passes to the liver, where it is stored. It is excreted partly in the bile and partly in the urine.

The irritant properties of copper sulphate are to some extent an asset. On ingestion vomiting occurs promptly and diarrhoea follows later. These processes aid in eliminating the poison from the system, so preventing absorption and reducing the risk of remote toxic effects on other organs. It has been observed (Smith and Cook 1934) that in non-fatal cases jaundice is sometimes a symptom, and this indicates that copper salts are apt to lead to liver damage. A considerable volume of experimental work has been done on this problem in the form of animal feeding experiments. Mallory, Parker, and Ny (1921) announced that it was possible to produce pigmentation and cirrhosis of the liver in rabbits and sheep by the oral administration of copper salts or of metallic copper in powdered form. Their results have been confirmed by Hall and Butt (1928), and denied by Flinn and Von Glahn (1929) and by Polson (1929), who claim that copper does not produce either pigmentation or cirrhosis, and that the pigmentation seen by Mallory and his co-workers is a natural phenomenon in the rabbit and is due to diet only. More recently Mallory and Parker (1931) have repeated their experiments and have found that copper given by injection in sufficient doses will kill a rabbit in from 24 hours to two to three weeks, and that necrosis and pigmentation of the liver cells can be demonstrated histologically. They assert that by special staining methods they have succeeded in demonstrating the copper in the liver cells. If the rabbits survive for a variable period, cirrhosis of the liver follows. They also describe the occurrence of necrosis of the tubular epithelium of the kidneys. Their results are supported by Hall and MacKay (1931), who found that 50% of their copper-fed rabbits developed cirrhosis of the liver, and that large quantities of copper were stored in this organ. Indirect support is also given by the finding of Gordon and Rabinowitch (1933) that in cirrhosis of the liver in man the copper content is increased. Thus there seems to be some evidence that copper salts can produce liver damage in addition to the gastro-intestinal irritation admitted by all toxicologists.

Manganese is generally regarded as being a relatively non-toxic element. A search of the literature has failed to reveal a case of acute manganese poisoning in man. There are, however, reports of chronic poisoning of industrial origin where the symptoms are those of hepato-lenticular degeneration. The neurological syndrome resembles in some respects that characteristic of Parkinson's disease (Goodman and Gilman, 1943). Von Oettingen (1935) reports that the lesions of the liver and central nervous system seen clinically can be produced in animals with toxic amounts of manganese, while Hurst and Hurst (1928) failed to detect any changes in the brain even in

the presence of gross damage to the liver. A single large dose of a manganese salt given subcutaneously will prove fatal in one to two days, while smaller doses repeated will produce cirrhotic changes in weeks or months; similar changes are found in rats which have had manganous chloride added to the diet (Findlay, 1924). Hurst and Hurst (1928) also produced acute and chronic changes after giving injections of manganese. It is fairly clear that both acute and chronic damage closely allied to acute yellow atrophy and cirrhosis, as seen in man, can be produced in animals experimentally.

It is questionable whether these experiments have proved the toxicity of manganese under ordinary conditions in man. According to Richards (1930) the bulk of the evidence seems to show that when ingested, even in fairly large amounts, manganese compounds have no toxic effects. He quotes the work of Reiman and Minot (1920) and of von Oettingen and Sollman to prove that feeding manganese ores to dogs and pigeons over a long period and in large amounts fails to produce any significant changes in the manganese content of the blood and tissues or any pathological symptoms. Richards fed manganese to pigs and found no toxic symptoms after the daily ingestion of 3.5 g. of manganese citrate for nearly nine months.

Case 1

A healthy boy aged 3 years 3 months took a box of tablets off the kitchen table in his home at 12 noon on April 23, 1946. According to the mother's estimate the box contained about 50 tablets. At 12.30 p.m. the same day the box was found to be empty, and the child admitted having swallowed all the tablets. Each tablet contained ferrous sulphate exsic. 3 gr. (0.2 g.), copper sulphate 1/25 gr. (2.6 mg.), and manganese sulphate 1/25 gr. Shortly afterwards the boy vomited and a few tablets were returned. During the afternoon of that day the child slept fitfully, was thirsty, and appeared to be very weak. At 6 p.m. he vomited again, and the vomitus was clear fluid only. He had a fairly comfortable night, and next morning his general condition had improved. On the following day he showed no symptoms likely to cause alarm till 10 a.m., when his skin became yellow, his pupils dilated, and he was very restless. The child's condition steadily deteriorated till 5.30 p.m. on April 25, when he died—53 hours after taking the tablets. Medical advice was sought by the boy's mother immediately she discovered what he had done, but no treatment was considered necessary in view of the fact that he had vomited. Actually he was not seen by a doctor till 48 hours afterwards and he had no treatment during the illness.

Post-mortem Examination.—The only significant external findings were a suggestion of jaundice in the sclerotics and some abdominal distension. The stomach contained 3 oz. (75 g.) of dark coffee-ground material and the mucous membrane along the lesser curvature was brown and necrotic. The remainder of the mucosa was rather oedematous but not acutely inflamed. The anterior wall of the stomach was stained blue and the subperitoneal vessels were injected. The small bowel was filled with black semi-solid material which had stained the rather oedematous mucous membrane, and there was vascular engorgement here also. The large bowel was healthy, but contained hard black masses of constipated faeces. The liver looked about normal in size, weighed 510 g., was not unduly flabby, and there was no pronounced wrinkling of the capsule. Both on the surface and on section this organ was in part bright yellow and in part reddish purple. The distribution of these areas was irregular and the normal liver markings had disappeared. The spleen was slightly enlarged, and there was a very small quantity of blood-stained fluid in the peritoneal cavity. The kidneys were in a state of advanced cloudy swelling, and in the pelvis of each there was a small quantity of bright-yellow crystalline material. The bladder contained 1/2 oz. (14 ml.) of cloudy urine which was not grossly bile-stained. The only abnormalities noted in the respiratory system were a few haemorrhages, each about 1/4 in. (0.6 cm.) in diameter, at the lung roots and some thick mucus in the bronchi. The heart muscle was pale and there were two small subendocardial haemorrhages on the posterior wall of the left ventricle. Further haemorrhages, similar to those seen on the lungs, were noted at the lower pole of the thymus and along the descending thoracic aorta. All the other organs were normal.

Histology.—The liver showed degenerative changes ranging from cloudy swelling to complete necrosis. Some of the liver lobules had entirely disappeared, while in others the central cells still remained. Where the liver cells had vanished the capillaries were widely dilated and there were extensive areas of haemorrhage. General "polymorph" infiltration was in evidence, and deposits of granular pigment were scattered about. There was necrosis of the gastric mucosa to varying depths. Throughout the stomach wall the vessels were intensely engorged and there were haemorrhages between the muscle layers. The submucous layer was infiltrated with "polymorphs," and in places there were minute abscesses. The tubules of the kidneys and the heart muscle showed cloudy swelling. The lungs were acutely congested and there was some oedema. Desquamated epithelium and red cells were present in the bronchi.

Chemical Analysis.—The liver and the bowel and its contents were wet-ashed with nitric and sulphuric acids. The copper in the residue was determined polarographically, using a Tinsley recording polarograph, with the following results: liver 11.2 mg., bowel 5 mg. The manganese was determined by converting it to permanganate ion and measuring the absorption in a Hilger-Spekker absorptiometer. The following results were obtained: liver, 4.2 mg.; bowel, 8 mg.

Case 2

At 7.15 p.m. on Sept. 9, 1946, a 1-year-old boy swallowed a quantity of the same proprietary preparation as in Case 1. It is estimated that he took between 30 and 35 tablets. The mother at once gave him salt and water, and when this failed to produce emesis she inserted her fingers into his throat and he vomited undigested food and a number of the tablets. Shortly afterwards the boy returned some brown material, and within an hour fresh and clotted blood. The child was admitted to hospital 90 minutes after taking the tablets.

On admission he was pale, collapsed, and shocked, with laboured, noisy, moist, and bubbly breathing. The pulse was thin and rapid, the rate being 170 a minute. There were dark-brown stains on his mouth resembling dried altered blood. The percussion note of his chest was unimpaired and moist breath sounds were heard at all areas. All other systems appeared to be normal. On admission to the ward the child started retching and when held up by his feet he vomited about 1 oz. (28 ml.) of fresh bright-red blood mixed with mucus. Immediate treatment was given to counteract the shock, warmth being applied externally. Gastric lavage was considered to be contraindicated, and bland fluids were given in the shape of milk and iced water. His general condition improved, and after a minim (0.06 ml.) of nepenthe at 10.15 p.m. he went to sleep. Four hours after admission the child again collapsed and appeared *in extremis*. The only positive findings were moist sounds in the chest and indications that the bronchial tree was full of fluid—presumably aspirated vomit. Intranasal oxygen was given, with slight improvement. The tablets in question were found to be radio-opaque, and the neck, chest, and abdomen were radiographed to determine whether any tablets could be seen in the stomach, bowel, or respiratory passages. None was observed. Atropine 1/150 gr. (0.43 mg.) was given at 4.5 a.m. on Sept. 10 and the child seemed slightly improved, but during the forenoon his temperature rose to 103° F. (39.4° C.). On the ground that an aspiration pneumonia was developing, a course of penicillin was started at 12 noon, with the result that the temperature began to fall. During the day there was one bowel action, the stool being very dark brown and offensive. At 6 p.m. the child again collapsed and vomited a small quantity of reddish-brown fluid. He was placed in an oxygen tent, but he died at 1.30 a.m. on Sept. 11—that is, about 30 hours after taking the tablets.

Post-mortem Examination.—This was carried out 34 hours after death. There was no jaundice. The only positive finding externally was the presence of a blotchy rash on the abdominal wall. The trachea and bronchi were filled with thick greenish

fluid which, from its colour, obviously contained some of the pigmented coating of the tablets. Both lungs were congested, and in them there were areas of collapse and a few scattered small haemorrhages. There were a few areas of pneumonic consolidation in the lower lobe of the right lung. The stomach was empty. Under the peritoneum covering it some haemorrhages could be seen. The lining of the stomach was brown, due to necrosis of the mucous membrane. The small bowel was normal, apart from an occasional area where the vessels were engorged. The large bowel was healthy and the contents of the bowel were stained black. The liver weighed 354 g. and its capsule was smooth. The liver tissue was yellow, but there were no haemorrhagic areas. Cloudy swelling of the kidneys was present. The urine contained no bile and no leucine or tyrosine crystals. The other organs were free from abnormality.

Histology.—The liver showed cloudy swelling and some fatty degeneration, but no necrosis. The gastric mucosa was necrotic to various depths, and much of the necrotic lining had been shed. The whole wall was intensely congested and there were extensive areas of haemorrhage in all its layers. In the submucous layer accumulations of "polymorphs" could be seen. The sections of the lung showed a typical bronchopneumonia. Cloudy swelling of the pancreas and kidneys was noted.

Chemical Analysis.—The liver and the bowel and its contents were analysed by the same method as was used in Case 1, with the following results: copper in liver, 2.88 mg.; in bowel, 4.58 mg.; manganese in liver, 1.375 mg.; in bowel, 3.56 mg.

Comment on Analysis

Quite a number of estimations of the normal copper content of the liver have been made, and a few of those published have been summarized in Table I. Many of the figures are given in terms of milligrams of copper per kilogram of dried tissue. The human liver contains approximately 75% of water (Gordon and Rabinowitch, 1933), and on this basis the figures quoted for dry tissue have been converted to milligrams of copper per kilogram of fresh liver. These figures are shown in parenthesis in Table I. It is at once apparent that there is a considerable variability in the results. This is due to two factors. First, the series of estimations was in most cases too short to strike a reliable average, as in any biological variable there is considerable deviation on either side of the mean; and, secondly, the copper content of the foetal and infant liver is considerably in excess of that of the adult (Sheldon and Ramage, 1931). From the figures quoted the average for infants up to 2 years is 40.4 mg. per kg. of fresh liver; for children from 2 to 14 years, 11.8 mg. per kg.; and for adults 7.2 mg. per kg.

TABLE I.—*Normal Copper Content of Liver*

Authority	Age	Copper per kg. of Liver: Fresh	Copper per kg. of Liver: Dry
		mg.	mg.
Sheldon and Ramage (1931)	Foetus	(37·5)	150
	Adult	(11·2)	45
Lesné, Zizine, and Briskas (1936)	Infants under 2 years	14·0	—
	Children 2–14 years	11·5	—
Cunningham (1931)	Adult	(6·2)	24·9
Brückmann and Zondek (1939)	Infants to 6 weeks	(57·5)	230
	Adults	(8·65)	34·6
Cited by Brückmann and Zondek:			
Ramage *et al.* (1933)	Infants to 7 weeks	(66·2)	265
	Children 3–12 years	(15·0)	60
Kleinmann and Klinke (1930)	Adults	(6·9)	27·5
Herkel (1930)	"	(6·4)	25·4
Tompsett (1935)	"	(5·5)	22·0
Morrison and Nash (1930)	Children 3–12 years	9·03	—
	Infants to 2 years	24·0	—
	Adults	5·86	—

In Table II some estimations of the manganese content of the liver are quoted. It seems that this element is present in fairly constant amounts, and that there is no storage in infancy (Brückmann and Zondek, 1939). The average content per kilogram of fresh liver is 1.8 mg.

TABLE II.—*Normal Manganese Content of Liver*

Authority	Manganese per kg. of Liver: Fresh	Manganese per kg. of Liver: Dry
Brückmann and Zondek (1939)	(1·75 mg.)	7·0 mg.
Cited by Brückmann and Zondek:		
Ramage *et al.* (1933)	(2·1 mg.)	8·4 mg.
Richards (1930)	1·75 mg.	—
Reiman and Minot (1920)	1·70 mg.	—

Table III shows the content of manganese and copper per kilogram of fresh liver in the two cases under consideration. There seems to be no parallel between the two. In Case 1 the liver contains about twice the amount of copper expected, and in Case 2 only a fifth of the normal average. In Case 1 the manganese in the liver is over four times the normal, while in Case 2 it is only twice. No reasonable conclusions regarding the passage of the absorbed copper to the liver can be drawn, because of the relatively small amounts ingested and the variability in the normal content in young children. In both instances the manganese content was substantially increased, which suggests that, as the basic figure is more constant, this element tends to pass to the liver.

TABLE III.—*Manganese and Copper Content of Liver in Cases 1 and 2*

	Liver Weight	Copper per kg. of Liver	Manganese per kg. of Liver
Case 1	510 g.	21·9 mg.	8·2 mg.
" 2	354 g.	8·1 mg.	3·9 mg.

Animal Experiments

In order to determine with certainty which of the ingredients of the preparation in question was responsible for the death of these two children, a number of animal feeding experiments were undertaken. Guinea-pigs and cats were used. In the first instance six pairs of guinea-pigs were treated with the tablets. One pair served as controls and were given 6 ml. of water only. The remainder were dealt with in pairs with 5, 4, 3, 2, and 1 tablet respectively. Those given two tablets at 3 p.m. one day were all found dead next day at 9.30 a.m. The post-mortem findings were similar in all cases. The stomach showed a bluish-green patch on the greater curvature, and was distended with granular coffee-ground material heavily stained with fresh blood. The mucosa was brown and necrotic, and patches of it had been shed. Haemorrhages could be seen with the naked eye in the stomach wall. The upper part of the small bowel was injected and the contents were blood-stained. The large bowel and its contents were normal, and the animals did not suffer from diarrhoea. The liver appeared normal and no abnormalities, apart from occasional haemorrhages on the lungs and pericardium, were noted elsewhere.

Histological examination of the stomach showed necrosis of the mucous membrane to varying depths, with detachment of the more superficial layers. The vessels were engorged and haemorrhages could be seen in the submucous and muscular layers. "Polymorph" infiltration of the submucosa was noted. No wholesale necrosis was seen in the sections of the liver, the commonest appearance being cloudy swelling. Some vacuolation of the cells was not uncommon, and this was more in evidence in those animals given the larger doses. Here the cytoplasm appeared granular and fragmentary, and sometimes the cell contained a nucleus isolated in a large vacuole surrounded by an intact cell membrane. These areas were irregularly scattered and did not bear any special relation to the portal canals. "Polymorph" accumulations in the liver sinuses were not uncommon, but the groups usually

amounted to no more than a half-dozen cells. No significant histological changes were observed in any of the other tissues.

The two controls and the two guinea-pigs given one tablet each remained apparently unaffected. One control and one of the other animals died later from bronchopneumonia. All four animals were dissected, and no abnormality was discovered in the gastro-intestinal tract.

Two cats were each given five tablets, and within a short time they became ill and vomited blood. One of the cats was killed 4¼ hours later. The other cat survived but was ill for several days. It refused food, had no energy, and its coat was ragged. It had apparently completely recovered 18 days later when it was destroyed. The post-mortem examination of the first cat revealed naked-eye and microscopical changes in the stomach identical with those found in the guinea-pigs. All the other organs, including the liver, were normal. The second cat appeared to be perfectly healthy at necropsy, and there was no histological abnormality of the stomach. Sections of the liver, however, showed changes similar to those seen in the guinea-pigs given the heavier dosage. Many areas looked healthy, while in others the cells were in various stages of degeneration up to complete necrosis. There were no large areas of necrosis, but rather small nests of cells here and there surrounded by healthy liver tissue. No regenerative processes were seen.

At this stage of the investigation it was apparent that when a certain dose of this preparation was exceeded it was relatively lethal to cats and guinea-pigs. To determine which ingredient was the noxious one, it was decided to administer them separately to a further batch of animals. To begin with, ferrous sulphate was used alone. This was given to four pairs of guinea-pigs in doses of 3 gr. (0.2 g.) each to the first pair, 6 gr. (0.4 g.) to the second, 9 gr. (0.6 g.) to the third, and 12 gr. (0.8 g.) to the fourth. One of the guinea-pigs given 3 gr. died in 5 hours, the other survived, as did the pair given 6 gr., while those given 9 and 12 gr. died overnight. The post-mortem findings were identical to those observed in the guinea-pigs previously treated with the proprietary tablets. The animal killed by 3 gr. of ferrous sulphate weighed only 210 g. while its mate weighed 445 g.; the pair given 6 gr. weighed 675 and 770 g. On considering those guinea-pigs killed by the smaller doses we find that, on an average, 1 gr. (0.065 g.) of ferrous sulphate per 64 g. body weight will prove fatal (Table IV).

TABLE IV

Weight of Guinea-pig	Dose of Ferrous Sulphate	Guinea-pig Weight per Grain of Ferrous Sulphate
416 g.	6 gr. (0·4 g.)	69 g.
355 g.	6 gr. (0·4 g.)	59 g.
210 g.	3 gr. (0·2 g.)	70 g.
535 g.	9 gr. (0·6 g.)	60 g.
560 g.	9 gr. (0·6 g.)	62 g.
	Mean:	64

The fact that the ferrous sulphate alone had the same effect and produced pathological changes identical with those occasioned by the proprietary tablets suggests strongly that the iron salt is the noxious ingredient. It was therefore decided to give six fresh guinea-pigs manganese sulphate and copper sulphate together. The proprietary tablets contain 1/75 gr. (0.87 mg.) of these salts for each grain of ferrous sulphate, and it was found that about 1 gr. of ferrous sulphate per 64 g. body weight of guinea-pig would prove fatal. The first pair were given 1/75 gr. of the manganese and copper salts per 64 g. body weight, the second pair double that amount, and the third pair a triple dose. This treatment had no effect of any sort on the guinea-pigs. It would appear, therefore, that the two children and the experimental animals died from acute ferrous sulphate poisoning.

Conclusions

The proprietary preparation in question is widely used therapeutically and is generally regarded as being quite innocuous. This may be true in ordinary doses, but the two cases described and the results of the animal experiments clearly show that in very large doses this preparation may be highly dangerous. It is clear that these are cases of acute ferrous sulphate poisoning. This salt, in contact with the gastric juice, would be converted into the chloride, which has a considerable irritant action. This accounts for the acute haemorrhagic gastritis found in the two children and in the animals. The remarkable feature of Case 1 was the extreme liver damage found. We failed to produce comparable lesions in the experimental animals. Of course, in their case death occurred quickly, while the elder boy lived for 53 hours after taking the tablets. This allowed time for considerable toxic absorption from the damaged tissues of the stomach, and this alone may have been sufficient to produce the degree of liver destruction found. The younger boy lived for 30 hours, and in his case the liver damage was not nearly so great. He died from an aspiration pneumonia, and had he not contracted this he might well have recovered. The quantities of copper and manganese taken were too minute to have any toxic effect.

Summary

The toxicology of the salts of iron, copper, and manganese is briefly reviewed.

Two cases of fatal acute poisoning due to a proprietary preparation containing ferrous sulphate, manganese sulphate, and copper sulphate are described.

The results of a chemical analysis of the liver and of the bowel and its contents are given in each case.

A short series of animal feeding experiments is described, proving that the ferrous sulphate is the noxious ingredient in the preparation concerned.

I have pleasure in acknowledging the help I have received in this investigation. Mr. R. Belcher and Mr. G. W. C. Milner, of the Department of Fuel Technology of Sheffield University, very kindly performed the chemical analysis of the organs. Dr. Beryl Smith, a resident physician at the Children's Hospital, Sheffield, provided me with the clinical report on Case 2. Dr. I. F. S. Mackay, Lecturer in Physiology at Sheffield University, undertook the feeding of the experimental animals. To these colleagues I am deeply indebted for their kindness and willing co-operation.

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FERROUS SULPHATE POISONING CAUSING PYLORIC OBSTRUCTION*

BY ISABELLA FORSHALL and P. P. RICKHAM, LIVERPOOL.

NUMEROUS reports of ferrous sulphate poisoning in children have been published in the last few years. The Royal Liverpool Children's Hospital and Alder Hey are the two major children's hospitals on Merseyside; during 1950–51, 12 children suffering from ferrous sulphate poisoning were seen at these hospitals.

Pyloric obstruction following the ingestion of caustics is a well-known complication; it has not, however, been recorded after ferrous sulphate poisoning. The two following cases in which the history and clinical, radiological, and operative findings were very similar are considered of sufficient interest to justify record.

CASE REPORTS

Case 1.—Marjorie H., 17 months of age, was admitted to Alder Hey Children's Hospital on May 28, 1949. Forty-eight hours previously she had swallowed between ten and fifteen Fersolate tablets. Following this accident she was 'off colour', refused to swallow solids, and screamed at night with abdominal pain. She had vomited four times shortly before admission; the last vomitus had contained black material.

On examination she was pale and apathetic; her pulse was 140 and of poor volume. Nothing abnormal was discovered on clinical examination. There were no petechiæ and the liver and spleen were not palpable. The bleeding time was 2 minutes and the hæmoglobin 98 per cent. She vomited fresh blood twice on the evening of admission and a blood transfusion was given. The next day (May 29) she had five more hæmatemeses and a further 300 c.c. of blood were transfused, after which her condition improved. Oral feeding was started, glucose-saline being given initially and then iced milk. On May 30 her general condition was satisfactory; hæmoglobin 101 per cent, but she continued to vomit brown material which gave a strongly positive reaction for blood and a weak positive reaction for ferrous iron. Vomiting slowly subsided and by June 1 she was taking a light diet. She made a satisfactory recovery and was discharged quite fit on June 10, no vomiting having occurred for ten days.

She was re-admitted on July 13 with the history that after discharge from hospital she had at first vomited once daily. During the week preceding re-admission, the frequency of the vomiting, which had no definite relationship to food, had increased to 2–3 times a day. The mother had noticed 'waves' passing over the abdomen from left to right.

ON EXAMINATION.—She was a thin, ill, dehydrated child; the tongue was furred and dry. There was marked fullness in the epigastric region and large peristaltic waves were seen crossing from the left costal margin towards the right hypogastrium. X-ray examination after a barium meal showed a grossly dilated stomach; after six hours only a trickle of barium had passed the pylorus (*Fig.* 461). It was feared that the barium would completely obstruct the pylorus and the stomach was therefore washed out; nevertheless, there was still barium in the stomach 24 hours later. The child was

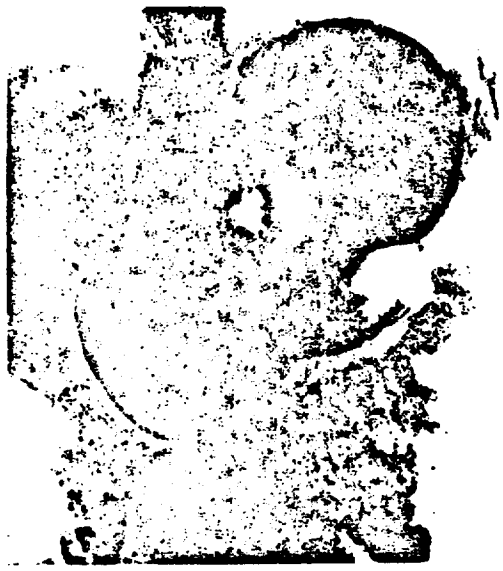

FIG. 461.—Radiograph of stomach three hours after ingestion of barium meal. The stomach empties slowly, the barium trickling through the narrow pyloric channel. (Case 1.)

put on continuous gastric suction and intravenous infusion of half-strength plasma.

AT OPERATION (July 15). The peritoneal cavity was entered through a midline supra-umbilical incision. The stomach was found to be dilated and thick walled; the pylorus was normal in appearance, but palpation gave the impression of thickening of the walls of the pyloric canal. Incision of the pylorus antrum anteriorly showed the gastric mucosa to be thick and œdematous and the pyloric canal grossly stenosed, only admitting a fine probe; no area of scarring of the mucosa of the pyloric antrum was identified. A posterior no-loop gastro-jejunostomy was performed.

Post-operatively the child was put on continuous gastric suction and intravenous glucose-saline infusion

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reduction in the size of the stomach and rapid emptying into the jejunal loop. The child was discharged on Sept. 10, at which time there was still a marked degree of epigastric distension.

She was followed up in the out-patient department, her abdominal distension gradually disappeared, and although she has remained thin and small (she was 54½ in. tall and weighed 30 lb. at 4 years of age) she has enjoyed good health, eats well, and is not constipated.

Case 2.—James M., 13 months of age, was admitted to the Royal Liverpool Children's Hospital on Jan. 13, 1952; he had swallowed an unknown number of Fersolate tablets two hours previously. Vomiting had occurred 15 minutes, and again 45 minutes, after the accident.

On examination, the child was pale, shocked, and drowsy; the pulse was 180 and of poor volume; the temperature 96 F. He vomited again and a gastric lavage with sodium bicarbonate solution was performed. An intravenous infusion of half-strength plasma was instituted and bismuth carbonate gr. 3 given at four-hourly intervals. The next day his condition had improved and there had been no further vomiting. There was vague tenderness over the epigastrium; the liver was not clinically enlarged. On Jan. 15 oral feeds were started; he vomited again once the next day. The intravenous infusion was discontinued on Jan. 17 as there was no further vomiting and the child was taking satisfactorily by mouth. He was discharged home on Feb. 1.

James was re-admitted on Feb. 5 with the history of having vomited after every feed since his discharge from hospital. The general condition was quite good and there was no dehydration. The child took feeds well but had several attacks of vomiting during the next few days. On Feb. 13 a barium meal was given and the child was screened; the barium passed rapidly into the stomach, which was of normal size. There was a small gastric diverticulum of the upper third of the greater curvature (*Fig.* 462). The stomach emptied very slowly, the barium trickling through a much narrowed pyloric channel (*Fig.* 463); there was still barium in the stomach after eight hours. On Feb. 20 a test feed was given and gastric peristaltic waves were seen crossing the epigastrium.

FIG. 462.—Radiograph of stomach after ingestion of barium meal. Dilated stomach with a small gastric diverticulum of the upper third of the greater curvature. *Case* 2.

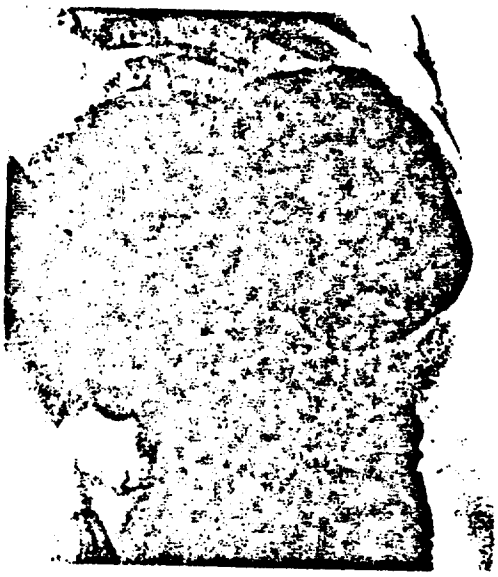

FIG. 463.—Radiograph of stomach six hours after ingestion of a barium meal. A grossly dilated stomach; only a trickle of barium has passed the pylorus. *Case* 2.

AT OPERATION (Feb. 27).—The peritoneal cavity was entered through a supra-umbilical transverse incision. The stomach was found to be dilated but its walls were not thickened; the pylorus looked normal, but its walls were definitely thickened to palpation. There was a small diverticulum, 1 in. in diameter about two-thirds up the greater curvature. A longitudinal incision was made along the anterior aspect of the pyloric antrum; a small probe could just be passed through the narrowed pylorus. It was seen that the pylorus was obstructed by the scar of a healed ulcer situated on the inferior, posterior, and superior aspects of the proximal end of the pyloric canal. The scar measured about 1 in. in length and ½ in. across. A Heinicke-Mikulicz pyloroplasty was performed, the diverticulum was excised, and the abdomen closed; 150 c.c. of blood were given during the operation. Continuous gastric suction and intravenous therapy were continued for twelve hours after operation. Fluids by mouth were then started. The child made an uninterrupted recovery.

DISCUSSION

Since Forbes (1947) and Thomsen (1947) each reported the death of a child following the ingestion of ferrous sulphate tablets, there have been many reports of ferrous sulphate poisoning in British and American journals.

The clinical picture of the acute phase of ferrous sulphate poisoning in children is now well known. Pallor, tachycardia, retching, vomiting, drowsiness, listlessness, cold clammy skin, often hæmatemesis and sometimes diarrhœa, the stools being either bloody or clear, have been described by many pædiatricians. Spencer (1951) stressed the point that symptoms may not appear for 24 hours after swallowing the poison, collapse being delayed. A number of necropsy findings have been described

in the literature; the stomach is most often affected and the small intestine less frequently. The mucosa is œdematous and congested and there are many hæmorrhagic and necrotic areas. These changes have been explained by Swift, Cefalu, and Rubell (1952) as follows: The caustic effect of the iron produces mucosal necrosis; this destroys the tissue barrier to iron absorption and permits chemically unaltered ferrous sulphate to gain entry into the veins and lymphatics, and causes thrombosis and gangrene.

There has been some disagreement as to the cause of death in these children. Prain (1949) has advanced the theory that death is caused by liver failure, but this is not substantiated or generally accepted.

Pyloric obstruction following the swallowing of corrosives is not uncommon. The first case was reported by Robert in 1828 and Strode and Dean (1950) collected 150 cases from the literature. Gray and Holmes (1948) in a comprehensive article listed the following chemicals as causes of pyloric stenosis: hydrochloric acid, sulphuric acid, nitric acid, trichloracetic acid, carbolic acid, zinc chloride, copper-sulphate, potash, and formaldehyde. McLanahan (1934) described a case caused by tincture of iodine.

In only 6·5 per cent of the 139 cases reviewed by Gray and Holmes (1948) was there a concommitant œsophageal stenosis. It is noteworthy how often the pyloric antrum and the pylorus are the only areas affected by the ingested caustic. This is explained by the rapid transit of ingested fluids along the *Magenstrasse* of the stomach (Waldeyer, 1908). Schulenburg (1941) quotes Testa (1938), who added caustic soda to a barium mixture and fed it to experimental animals, following the progress of this mixture under the X-ray screen. The fluid passed quickly down the œsophagus and along the *Magenstrasse* and was held up in the pyloric antrum by firm spasm of the pylorus. Subsequent necropsy showed that the trauma to gastric mucosa was confined to the pyloric area.

In cases of pyloric obstruction following the ingestion of caustics symptoms do not manifest themselves until between ten days and six weeks after the accident (Schulenburg, 1941). Presumably the fibrosis and narrowing of the pylorus caused by healing of the traumatic ulcer becomes severe enough to produce symptoms only after this period has elapsed. The two cases reported in this paper show a time lag within these limits (46 and 23 days respectively).

In our first case we did not slit open the pyloric antrum and pyloric canal and could therefore not see the ulcer scar which caused the obstruction. The scar of the ulcer was clearly demonstrated in the second patient.

Milroy Paul (1951), in discussing the treatment of pyloric obstruction following corrosive poisoning, states that although partial gastrectomy has been advocated, only 4 of the cases reported in the literature have in fact been treated by this operation. The majority of patients have had a gastro-enterostomy performed, with excellent results. The first case in which a successful gastro-enterostomy was performed for pyloric stenosis due to a corrosive was in 1884 when Monastyrski successfully treated a 36-year-old woman who had ingested sulphuric acid. As children under 5 years do not stand partial gastrectomy well, a gastro-enterostomy was performed on our first patient. In the second child we widely opened the pyloric antrum and pyloric canal in order to inspect the mucosal lining and it then became obvious that a Mikulicz pyloroplasty could easily be performed. Pyloroplasty has been criticized as a treatment of pyloric stenosis secondary to corrosive poisoning, but several successes are reported in the literature, amongst them the first successful pyloroplasty ever to be performed by von Mikulicz in 1888 on a 20-year-old girl who gave a history of having drunk large quantities of vinegar many years previously.

The presence of a gastric diverticulum in the second patient raises the question if this was an incidental finding or secondary to the pyloric obstruction. Only 8 cases of gastric diverticula in infancy have been described in the literature. Ogur and Kolarsic (1951) reported a child of 9 weeks of age with congenital pyloric stenosis; at operation a wide diverticulum on the lesser curvature, 1 cm. proximal to the pylorus, was found. This was regarded as a pulsion diverticulum secondary to an obstruction of nine weeks' duration. In our case the site of the diverticulum and its histological appearance are in favour of a congenital origin.

SUMMARY

Two cases of pyloric stenosis in young children, following the ingestion of Fersolate tablets, have been described. The history and clinical findings in the two cases showed marked similarity. Both were successfully treated surgically. It is believed that these are the first two cases to be recorded.

We would like to express our thanks to Dr. R. W. Brookfield for referring *Case* 2.

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DEATH FOLLOWING INGESTION OF FERROUS SULFATE*

F. H. FOUCAR, M.D., BENJAMIN S. GORDON, M.D., AND
SIDNEY KAYE, M.S.

From the First Army Medical Laboratory, New York, N. Y.

Ferrous sulfate is regarded as a relatively nontoxic substance. Reports of poisoning with iron salts are scarce. Helpern[7] in 1937 reported 898 deaths from accidental, homicidal and suicidal poisonings, but no case of poisoning from iron salt was included. Several cases of ferrous sulfate poisoning have been reported [1, 2, 4-6, 8] in both foreign and American journals during the period from 1850 to 1890. Peterson, Haines and Webster[9] refer to a death after ingestion of 45 cc. of tincture of iron, equivalent to 6 Gm. of salt, but make no reference to the pathologic findings. They also mention four cases of homicidal poisoning in Martinique with ferric chloride. Necropsy of one case showed a greenish black fur-like "mud" covering the tongue, esophagus and stomach; swelling, congestion and ecchymotic points in the liver and kidneys, and marked hyperemia of the brain and membranes.

The pharmacologic actions of iron have been studied extensively, but studies on the toxicologic effects of iron salts are meager. Edmunds and Gunn in Cushny's Pharmacology[3] describe the effects of oral ingestion of large quantities of iron salts as consisting of "pain and uneasiness in the stomach, nausea, vomiting and often purging, with all the ordinary symptoms of gastrointestinal irritation. General weakness and collapse may be induced, but are manifestly secondary to the gastrointestinal irritation; and no symptoms which may be attributed to the absorption of iron have been observed in either man or animals." We have had an opportunity to study a case in which ferrous sulfate was ingested, and we were able to confirm the above findings that death resulted from secondary shock following the ingestion of a strong corrosive agent. Iron was important in this case only as the vehicle of an anion that constituted a strong acid.

REPORT OF CASE

Clinical data. A white male, age 26, was admitted to the hospital in shock with a history of having accidentally ingested one-quarter (¼) pound of ferrous sulfate U.S.P. in aqueous suspension. He was cyanotic, had vomited blood and was doubled up with abdominal pain. The skin presented cyanosis and purplish blotches. The scleral blood vessels were congested, and the pharynx was injected. There were blood stains about the mouth, nose and pharynx. Examination of the chest was negative. No arterial pulse was palpable, nor could a blood pressure reading be obtained. The heart was not enlarged to percussion, the sounds were heard faintly and the rate was about 130 per minute. The abdomen was not distended, but there was boardlike rigidity. No peristalsis was felt or heard. A dark brownish black liquid was oozing from the anus. The reflexes were normal. The red blood cell count was 7,860,000, hemoglobin 114 per cent, leukocyte count 55,750 with a differential count of neutrophils 74 per cent, lymphocytes 25 per cent and eosinophils 1 per

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cent. The *treatment* consisted of gastric lavage, oxygen, transfusion with whole blood and artificial respiration. The patient expired after about three hours.

Autopsy findings. The pupils were slightly dilated. The gums were discolored dark brown, and the nail beds were cyanotic. The muscles were of normal color. The peritoneum was dusky red and smooth, and there was a small amount of sanguineous fluid in the pelvic cavity. There were small, dark red, soft lymph nodes along the greater curvature of the stomach. There was a small amount of sanguineous fluid in the left pleural cavity. The pericardium was dusky red in color. Examination of the *lungs* revealed hemorrhage in the left lower lobe. The other lobes were edematous. The bronchi were congested and contained a frothy fluid. The *heart* showed subendocardial hemorrhage throughout the left ventricle. The *spleen* was grossly normal. The *liver*, *pancreas* and *adrenals* displayed no gross abnormalities. The *esophagus* showed erosions of the mucous membrane and a grayish substance adherent to the lining of the distal end. The *stomach* was dilated and filled with dark, bloody, thick fluid. The gastric wall showed large areas of hemorrhage. The rugae were obliterated and the mucous membrane was discolored gray and red, extensively eroded and covered with adherent grayish black, metallic, granular substance. Similar erosions and grayish black content were noted in the *duodenum* and upper portion of the *jejunum*. The mucous membrane of the rest of the intestinal tract was congested and covered with grayish black granular material. The large intestine contained reddish black fluid material, and the mucous membrane was covered with plaques of grayish black material. The *genitourinary* tract was normal on gross examination. The *dura* and *dural sinuses* were normal. The cerebral vessels were normal. The *brain* and ventricles were not remarkable.

Microscopic examination of the *lungs* showed a filling of the alveoli and bronchioles of the left lower lobe with whole blood. There was no pneumonic reaction. The epithelium of the trachea was desquamated. Sections of the *stomach* showed necrotic mucosa covered with a granular mantle. There was congestion, hemorrhage and edema, and lymphocytic infiltration throughout the substantia propria mucosae. The submucosa was congested. The epithelium of the *jejunum* and *ileum* showed necrosis with deposition of coarsely granular material. The submucosa presented congestion and edema. The *liver* showed varying degrees of acute parenchymatous degeneration. The cytoplasm was finely granular. Many cells were without nuclei while other liver cells displayed large hyperchromatic nuclei. The *spleen* displayed congestion and hemorrhage of the red pulp. The interlobular fatty areolar tissue of the *pancreas* presented edema and varying degrees of hemorrhage; no fat necrosis was identified. Sections of the *adrenals* were normal. The *kidney* sections showed congestion of the glomerular capillaries. The cells lining the convoluted tubules showed finely granular degeneration and many cells had no nuclei. The lumina of the convoluted tubules were small and contained a finely granular, acellular detritus. In the *glomerular* zones of the medullary rays (deep cortex), there were a few areas of interstitial lymphocytic infiltration. Examination of the sections of the *forebrain* showed subarachnoid congestion, edema of the cerebral cortex and pyknosis of the pyramidal cells. No hemorrhage and no perivascular cellular infiltrations were noted. The *basal ganglia* showed venous and capillary congestion. Sections of *medulla* displayed subependymal edema. There were no changes in the hypoglossal, vagal arcuate or inferior olivary nuclei, or in the *pituitary*.

Prussian blue reaction. (Ferrous sulfate is unstable and is oxidized to ferric sulfate.) The mantle attached to the surface of the gastric mucosa displayed a Prussian blue reaction. There was aspiration of gastric content in the lungs. The alveolar walls displayed a Prussian blue reaction involving the endothelium of the capillaries and the cytoplasm of included granulocytes. Sections of kidney, brain, thyroid, liver and adrenal all gave a negative Prussian blue test for ferric iron. The residue in drinking glass contained ferrous sulfate. The vomitus contained large amounts of ferrous ions, small amounts of ferric ions and large amounts of sulfate radical. The contents of the stomach and large intestine revealed large amounts of blood, ferrous and ferric ions and sulfate radical. The Prussian blue test was positive only in those tissues which were directly in contact with the ferrous sulfate.

SUMMARY

The oral ingestion of one-quarter pound of ferrous sulfate was followed by death within three hours. The symptoms were those of very severe gastrointestinal irritation, and death was attributed to shock. There was no clinical, pathologic, or toxicologic evidence of absorption of the ferrous sulfate.

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COMPARATIVE EFFECTIVENESS OF VARIOUS IRON COMPOUNDS IN PROMOTING IRON RETENTION AND HEMOGLOBIN REGENERATION BY ANEMIC RATS

SMITH FREEMAN AND MARIE W. BURRILL
WITH THE TECHNICAL ASSISTANCE OF MARGARET GRIESSER
Department of Physiology, Northwestern University Medical School, Chicago

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Iron compounds used for the enrichment of bread and flour should possess two qualifications. First they should be utilized by the body and secondly, they should have no adverse effect upon the preservation of flour (Fed. Reg., '41). Most compounds of iron that are readily soluble in water and dilute acid cause flour to become rancid or to have a decreased vitamin content (Gillet, '45). Sodium iron pyrophosphate seems to have no adverse effect on flour, but there is some difference of opinion as to the effectiveness of its utilization by the body. Nakamura and Mitchell ('43) reported a relatively high degree of utilization of this compound in anemic rats while Street ('43) found it to have only about half the effectiveness of ferrous sulphate in promoting hemoglobin regeneration in anemic rats.

The present study was undertaken to compare various iron compounds used for the enrichment of bread and flour as to their effect on iron retention and hemoglobin regeneration in anemic rats. The compounds were not only tested as such, but were also compared when used as the fortifying ingredient in specially prepared breads. Some observations were also made on the efficacy of two iron compounds in preventing anemia in milk-fed rats.

METHODS

Hemoglobin was determined by the method of Wu ('22) as adapted to a photoelectric colorimeter. The white blood cell pipettes used for hemoglobin estimations were calibrated on a standard blood of known iron content. The iron analyses by the thiocyanate method were done as previously described (Freeman and Ivy, '42) with only one modification. Immediately after extraction of the iron thiocyanate with iso-

amyl alcohol, the colorimeter tubes containing the extract were warmed in a water bath at 40°C. for 5 minutes and then read.

The rats used in the experiment were distributed among the various groups according to weight and sex so as to make the groups as uniform and comparable as possible.

Anemia was produced according to the method of Elvehjem and Kemmerer ('31) using cages that were described previously (Freeman and Ivy, '42). The rats were depleted on a milk diet until the hemoglobin concentration was approximately 3.0 gm. 100 ml. of blood. This degree of depletion usually required 35 to 45 days after weaning (21 days of age).

The depleted rats received the iron compounds under study as a supplement to the milk diet which was offered ad libitum. The iron compounds were mixed with cane sugar in such proportion that 1 gm. of the mixture contained 0.25 mg. of iron. The iron content of the mixture was verified by analysis. That amount of iron-sugar mixture providing 0.25 mg. of iron daily for 28 days (as determined by analysis) was divided into 28 capsules (no. 000). The contents of one capsule were fed daily in a clean salt cellar with added thiamine chloride (10 γ) and copper and manganese as sulphates (0.05 mg. of each). The daily supply of milk was withheld until the supplement was consumed.

The breads containing the various iron compounds were made from a dough of the following composition:[1] flour (unenriched) 100; water 65; yeast 2; salt 2; sugar 5; milk (dried skim) 3; yeast food 0.5; and lard 2.

To this basic mixture was added an amount of iron which would give 0.20 mg. of extra iron to each 5 gm. of air-dried bread or 18 mg. of extra iron per loaf of bread.[2] The iron salt was thoroughly mixed with the dry ingredients before the dough was prepared. The baked bread was sliced, air-dried, ground, mixed and analyzed. According to the analyses, the amount of dried ground bread which contained 0.27 mg. of iron was 4.5 to 5.0 gm. This amount of bread was fed daily to the experimental animals. The control rats received the same amount of the plain bread which contained 0.07 mg. of iron by analysis. Milk was offered to the animals only after the bread was completely consumed.

The rats were killed after 28 days on the supplemented diet. Hemoglobin determinations were made on the seventh, seventeenth and twenty-eighth days.

[1] Prepared by the American Institute of Baking.

[2] This amount of iron is slightly in excess of that recommended for the enrichment of bread and flour by the National Research Council. See Bull. Nat. Res. Council, no. 110, Nov. 1944, "Enrichment of Flour and Bread".

In the part of the study concerning prevention of anemia in milk-fed rats by iron compounds, the weanling rats (21 days old) were given 0.25 mg. of iron daily either as ferric chloride or sodium iron pyrophosphate in a sugar mixture similar to that described above. The control group received the sugar alone. Each day, after the sugar or sugar mixture was entirely consumed, the rats were given milk ad libitum. Hemoglobin values were determined at 15, 30 and 40 days, At 40 days the rats were killed and the carcasses analyzed for iron content. Hemoglobin and iron determinations were also made on 21-day-old rats that only had access to milk since the twelfth day of life.

RESULTS

The iron content of the carcass after 40 days of depletion was found to average 0.94 mg. per rat (see table 2). This value was taken as the iron content of all the depleted rats used in this study and the retention of iron from any supplement was calculated by subtracting 0.94 from the final iron content of the carcass. The total iron intake from bread or supplement divided into the iron retained by rats on the supplement times 100 gives the percentage retention from various sources. The relative iron retention was calculated by comparing retention from other sources with that from ferric chloride. The high relative retention of the iron contained in plain bread is in accord with the finding of Smith and Otis ('37), who showed that small amounts of iron result in a relatively greater hemoglobin regeneration by anemic rats. According to these same authors, the total daily amount of iron fed in these experiments is at the upper limits of the range over which there is a direct relation between hemoglobin regeneration and iron intake. The data reported here demonstrate a direct relation between iron retention and hemoglobin regeneration.

Iron retention and hemoglobin regeneration by anemic rats on the various iron salts when fed as such or contained in bread are presented in table 1. These data show a good correlation between hemoglobin concentration and the iron content of the carcass.

The rats which received ferric chloride with sugar or in bread showed the greatest iron retention and hemoglobin formation.

Iron retention and hemoglobin regeneration for sodium ferric orthophosphate, both as the salt and in bread, and for ferric orthophosphate in bread were only slightly less than for ferric chloride iron. Reduced iron was somewhat less effective, both as the salt and in bread. Sodium iron pyrophosphate was the least effective of the compounds studied. Doubling the daily intake of this salt was without significant effect upon iron retention or hemoglobin regeneration.

TABLE 1

Iron retention and hemoglobin formation by anemic rats receiving iron supplements or iron enriched bread.

GROUP NO.	SOURCE OF Fe [1]	NO. RATS IN GROUP	AVE. PERIOD OF DEPLETION	AVE. WT. AT START OF SUPPLEMENT	AVE. WT. GAIN ON SUPPLEMENT	AVE. Hb AT START OF SUPPLEMENT	AVE. Hb INCREASE ON SUPPLEMENT	C_1 [2]	AVE. TOTAL Fe CONTENT OF CARCASS	C_1 [2]	RELATIVE Hb REGENERATION IN %	RELATIVE RETENTION OF Fe	% Fe SUPPLEMENT RETAINED
			days	*gm.*	*gm.*	*gm./100 ml.*	*gm./100 ml.*		*mg.*			*%*	
1	Ferric chloride	10	34	83	102	3.07	10.65		5.83				
2	Ferric chloride	10	36	89	107	2.96 } 3.01	9.72 } 10.00		5.60 } 5.75				
3	Ferric chloride	9	38	93	91	3.00	9.47 ± 1.17		5.82 ± 0.70		100	100	68.0
4	Sodium iron pyrophosphate	10	35	85	92	3.04	4.76 ± 1.74	2.5	3.19 ± 0.42	3.7	48.0	46.6	32.1
5	"Double amount" sodium iron pyrophosphate	6	36	90	55	2.97	6.18 ± 1.50	2.5	2.91 ± 0.13	3.8	62.3	40.9	14.0
6	Reduced iron	10	34	84	107	2.92	8.85 ± 0.89	0.8	5.15 ± 0.59	0.6	83.4	87.5	59.3
7	Sodium ferric orthophosphate	10	39	94	88	2.97	9.26 ± 1.52	0.4	5.26 ± 0.59	0.5	93.5	89.9	61.0
8	$FeCl_3$ bread	10	39	94	86	2.92	9.28 ± 1.10		5.61 ± 0.62		100	100	61.0
9	Sodium iron pyrophosphate bread	10	34	91	83	3.00	4.09 ± 1.10	3.5	3.32 ± 0.69	2.7	44.5	51.0	30.8
10	Reduced iron bread	9	44	91	93	2.78	7.95 ± 1.64	0.7	4.86 ± 1.00	0.5	86 0	84.0	51.0
11	Ferric orthophosphate bread	9	42	92	107	3.06	8.30 ± 1.48	0.5	5.27 ± 0.74	0.4	90.5	92.7	56.5
12	Sodium ferric orthophosphate bread	9	45	94	98	2.86	8.66 ± 1.13	0.5	5.29 ± 0.73	0.3	93.2	93.1	56.7
13	Plain bread	11	40	92	69	2.92	2.38 ± 1.09	4.9	2.33 ± 0.36	6.4	25.9	29.8	68.0

[1] Source of iron compounds (1, 2, 3, 8) Mallinckrodt, (4, 5, 9, 12) Victor Chemical, (6, 10, 11) Merck & Co.

[2] C_1 = C.R. = critical ratio, considered significant when greater than 2 according to Fischer's rule.

There is good agreement between the results obtained when the iron salts were fed mixed with sugar and when contained in bread. The relative retention of iron from various sources is in the same order in either case. The absolute amounts retained from any given compound are so similar for iron contained in bread as compared to that mixed with sugar as to indicate that the absorption of iron was not significantly altered by its inclusion in the bread. Widdowson and McCance ('42) found that iron was absorbed by human subjects from a diet that contained 40–50% of its calories as white bread but that its absorption was reduced when white flour was replaced by one containing considerable quantities of bran.

TABLE 2

Prevention of anemia in milk-fed rats.

SUPPLEMENT	NO. RATS IN GROUP	TIME ON EXPERIMENT	INITIAL WT.	AVE. WT. GAIN ON SUPPLEMENT	AVE. Hb AT 15 DAYS OF SUPPLEMENT	AVERAGE FINAL Hb	AVE. Fe CONTENT OF CARCASS	Fe RETAINED[2]	RELATIVE RETENTION OF Fe	RELATIVE INCREASE IN HEMOGLOBIN
		days	*gm.*		*gm./100 ml.*	*gm./100 ml.*	*mg.*	*mg.*	%	%
$FeCl_3$ + sugar	7	40	30	62	13.60	13.08 ± 2.03	4.00 ± 0.97	3.06	100	100
Pyro[1] + sugar	7	40	28	80	7.87	7.20 ± 0.66	2.32 ± 0.3	1.38	45	41.5
Sugar	6	40	28	44	6.12	3.04 ± 0.72	0.943 ± 0.21			
21-day-old rats	12	0	35			9.40 ± 0.33	1.06 ± 0.14			

[1] Sodium iron pyrophosphate.

[2] Final Fe content of Fe supplemented group minus Fe content of group fed sugar alone.

A lower iron retention and hemoglobin regeneration by anemic rats fed sodium iron pyrophosphate was demonstrated in three separate experiments; first, with the compound fed at two concentrations, second, when added to bread and third when it failed to prevent the development of an anemia in rats (table 2). It does provide sufficient iron to permit growth but the retention of iron from this source was only approximately half that of ferric chloride, whether used in the treatment or prevention of anemia.

The various iron compounds show the following order of effectiveness in their relative retention by anemic rats: ferric chloride > sodium

ferric orthophosphate = ferric orthophosphate > reduced iron > sodium iron pyrophosphate (table 1). The order is the same whether the rats were fed the compounds themselves or received bread containing them. The relative degrees of hemoglobin regeneration for the compounds or enriched breads also give the same order whether determined after 7, 17 or 28 days. For this reason only the final hemoglobin values are included in the table.

Street ('43) studied hemoglobin regeneration in anemic rats and obtained results which indicate essentially the same relative utilization of ferrous sulfate and sodium iron pyrophosphate as that which we have obtained for ferric chloride and sodium iron pyrophosphate. The higher hemoglobin concentration on sodium iron pyrophosphate reported by Nakamura and Mitchell ('43) may be due to the relatively low weight gain of their rats during the experimental period. The degree of anemia was also less in their animals at the beginning of the experimental period. Iron retention was greater in 21 days for three sodium iron pyrophosphate rats reported by Nakamura and Mitchell than for our 28-day animals maintained on the same supplement, while ferric chloride retention was relatively greater in our animals.

The uniformity of iron retention and hemoglobin regeneration by anemic rats on a given compound is illustrated by the data obtained on three different groups of rats fed ferric chloride. These three groups were controls for rats fed sodium iron pyrophosphate, reduced iron and sodium ferric orthophosphate. The three groups were studied at different times and the rats were from different litters. There is good agreement among the three groups both as to iron retention and hemoglobin regeneration. Variation in hemoglobin regeneration is greater than for iron retention but the hemoglobin increase is also greater so that the impression derived from either determination is generally much the same. In studying the efficacy of a given iron compound as a source of iron, iron content appears to be a more direct measure than hemoglobin concentration. Other factors than iron absorption may affect hemoglobin regeneration and the concentration of hemoglobin in the blood is subjected to other factors that influence blood volume. The influence of growth on the concentration of hemoglobin in animals with a similar iron retention is well illustrated by groups 4 and 5, fed different amounts of sodium iron pyrophosphate (table 1). Although iron retention was essentially the same for the two groups, the hemoglobin increase of group 5 was on an average about 2 gm. higher while the weight gain of this group was only slightly more than half that of group 4.

In the prevention of anemia in weanling rats, the relative iron retention of ferric chloride and sodium iron pyrophosphate was similar to that obtained in the depleted rats (see table 2). So far as hemoglobin formation is concerned, this experiment is theoretically complicated by the fact that the diet is deficient in copper and manganese. If these two substances are supplied the development of anemia may be retarded, while if these substances are not supplied hemoglobin formation may be influenced by their absence as well as by the availability of iron. At the end of this experiment the variability both of hemoglobin and total iron content was greater for the rats fed ferric chloride than was the case at the end of the depletion experiments (see table 1).

The prevention of anemia in milk-fed rats offers a method of evaluating iron compounds which has certain desirable aspects. This procedure saves time since the experiment is ended by the time the control animals are depleted which is actually the starting point in the depletion experiments. Thus the prevention method covers a span of 40 days' time while the depletion method in our experience takes 60 to 70 days. During depletion some rats do not grow sufficiently or develop respiratory tract infections and have to be discarded as unfit for experimental material. This loss, representing considerable wasted time and effort, is reduced when the source of iron is fed from the time of weaning, only the control group being subjected to these hazards. The hemoglobin and total iron content of the twelve rats killed at weaning in the present experiment (table 2) indicate that the rats at this age were quite uniform when treated as described by Elvehjem and Kemmerer ('31).

SUMMARY

1. The retention of iron from different sources by anemic rats was qualitatively and quantitatively similar irrespective of whether the iron salts were fed as such or contained in bread.

2. The various iron compounds tested showed the following order of effectiveness with respect to the relative degree of iron retention and hemoglobin regeneration produced in anemic rats: ferric chloride > sodium ferric orthophosphate = ferric phosphate > reduced iron > sodium iron pyrophosphate.

3. Prevention of anemia in milk-fed rats given supplements of ferric chloride or sodium iron pyrophasphate for 40 days after weaning (21 days) gave results for relative iron retention and hemoglobin regeneration similar to those obtained with depleted rats fed the same supplements for 28 days.

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Biological Availability in Animals of Iron from Common Dietary Sources

James C. Fritz, Gwendolyn W. Pla, Talmadge Roberts, J. William Boehne, and Edwin L. Hove

Iron sources that are, or might be, used for fortification of feeds and foods were examined by the hemoglobin repletion technique with anemic chicks and rats. Similar results were obtained with each species. Reagent grade $FeSO_4 \cdot 7H_2O$ was used as the reference standard, and relative biological values for other iron sources were expressed as a percentage of the response to ferrous sulfate. Iron compounds studied were found to have relative values ranging from 0 to about 107. Food sources of iron were less well utilized than the more available inorganic iron sources. The influence of other dietary components was minor compared with the influence of the iron source. Provided that there was some availability, increased dietary levels of the poorly utilized iron sources were effective for the cure of iron-deficiency anemia.

Nutritional anemia is one of the most prevalent deficiency diseases in the U.S. (Finch *et al.*, 1968; Goldsmith, 1965; Gutelius, 1969; Schaefer, 1969) and throughout the world (Blanc *et al.*, 1968). The incidence is highest in young children and in women during their fertile years. Hemoglobin values below 10 g per 100 ml of blood, and hematocrit below 31.5% packed cell volume are considered abnormal by most investigators. There has been no improvement in the situation among children during the past 30 years (Gutelius, 1969). It is difficult to assess the effect of low hemoglobin and hematocrit values on the health of the affected individual. There is at least statistical evidence that anemic individuals have more frequent and more serious infectious diseases (Andelman and Sered, 1966; Bothwell and Finch, 1962; MacKay, 1928). A deficiency of dietary iron may lead to tissue depletion of iron-containing or iron-dependent enzymes and may cause secondary phenomena, including malabsorption (*Nutr. Rev.*, 1969). An iron-deficiency state may exist before reduced hemoglobin and hematocrit are apparent (Sood *et al.*, 1968).

Although anemia may result from many different causes, the form most frequently encountered is iron-deficiency anemia (Filer, 1969; Finch, 1969; Woodruff, 1969). The USDA's Agricultural Research Service (1969) estimates that, on the average, infants under 3 years of age and women under 55 years of age consume only about half of the recommended dietary allowance of iron (NAS, 1968).

Changing food habits have reduced the dietary intake of iron. Little food is cooked in iron pots that normally contribute substantial quantities of iron to the food (Peden, 1967). Another factor is the use of short extraction flour and other cereals. For example, Watt *et al.* (1963) note that while whole wheat flour contains about 33 mg of iron per kg, patent flour contains only about 8 mg. The cereal enrichment program aims to restore to the refined cereal products the whole grain levels of iron and certain B vitamins. The standards for enriched cereals (Code of Federal Regulations, 1969) state that the supplemental iron shall be a source that is harmless and assimilable. No criteria are given for determining whether or not the iron actually is assimilable.

Controversy has arisen over the effectiveness of different iron compounds that have been used for food fortification. Steinkamp *et al.* (1955) considered iron supplied as ferrous sulfate, reduced iron, ferric orthophosphate, and sodium iron pyrophosphate to be about equally effective, whereas others found more variation in the availability of iron from different sources (Ammerman *et al.*, 1967; Blumberg and Arnold, 1947; Freeman and Burrill, 1945; Hinton and Moran, 1967; Nakamura and Mitchell, 1943; Street, 1943). Hinton and Moran (1967) found considerable difference in availability of different samples of reduced iron. Harmon *et al.* (1967, 1968) found ferric ammonium citrate and ferrous sulfate to be about equally effective for preventing anemia in young pigs, but ferrous carbonate was less effective. Ammerman *et al.* (1969) showed that availability of ferrous carbonate was correlated with *in vitro* solubility. A "syrup" containing ferrous carbonate was reported to be an effective hematinic (Djafari and Kettler, 1969). In most cases where no apparent differences were found in availability of iron from various sources, the actual utilization of iron was very low by nonanemic individuals (Harrill *et al.*, 1957). This may give rise to erroneous conclusions.

Food sources of iron are less well utilized than many inorganic iron salts (Hussain *et al.*, 1965; Narula and Wadsworth, 1968; Underwood, 1962). Many factors are reported to influence the absorption of dietary iron (Brise, 1962; Brise and Hallberg, 1962; British Ministry of Health, 1968; Greenberg *et al.*, 1957; Greenberger and Ruppert, 1966; Herndon *et al.*, 1958; Kaufman *et al.*, 1966; Reddy *et al.*, 1965; Smith and Medlicott, 1944; Tucker *et al.*, 1957).

The purpose of this study was a critical examination of the biological availability of iron from various sources. Special attention was directed to iron compounds that are, or that might be, used for food fortification.

MATERIALS AND METHODS

The criteria used to judge availability of dietary iron were the repletion of hemoglobin and hematocrit in young chicks and rats made anemic on a low iron diet (Pla and Fritz, 1970). Most lots of basal diet contained about 7.2 mg of iron per kg. Reagent grade ferrous sulfate ($FeSO_4 \cdot 7H_2O$) was used as the reference standard, and the quantity of iron furnished by the sample was compared to the quantity of iron furnished by the ferrous sulfate that was required to produce the same response in terms of hemoglobin and packed cell volume. All comparisons were made at suboptimal levels of response.

Except when reagent grade chemicals were used at their theoretical iron content, samples were analyzed for iron content by the AOAC method (1965). They were then added to the test diets in quantities required to furnish the desired iron contribution to the diet. When only small quantities of supplement were required, the test samples replaced a small portion

Division of Nutrition, Food and Drug Administration, Department of Health, Education and Welfare, Washington, D.C. 20204

Table I. Comparison of Availability of Iron to Anemic Chicks and Anemic Rats

Iron Source	Relative Biological Values[a]	
	Chicks	Rats
Ferric ammonium citrate	115	98
Ferric orthophosphate #1	18	12
Ferric orthophosphate #2	9	12
Ferric orthophosphate #3	12	30
Ferric sulfate	65	100
Ferric oxide	4	6
Ferrous carbonate #1	2	1
Ferrous carbonate #2	2	0
Ferrous carbonate #3	6	0
Ferrous carbonate #4	2	2
Fish protein concentrate	22	53
Reduced iron #1	59	34
Reduced iron #2	41	16
Reduced iron #3	66	36
Reduced iron #4	43	37
Sodium iron pyrophosphate #1	2	11
Sodium iron pyrophosphate #2	13	19
Trace mineral mix (commercial)	14	21

[a] Relative biological value = (100 × mg Fe/kg from $FeSO_4$) / (mg Fe/kg from sample) to give equal curative effect.

of the whole basal diet. When large quantities of the samples were needed to furnish the desired level of supplemental iron, the samples were added in place of dried skim milk, degermed corn meal, and glucose monohydrate (singly or in combination) to maintain protein and energy levels of the test diets at comparable levels.

Hemoglobin was determined by the method of Crosby *et al.* (1954) and hematocrit was determined as described by Cohen (1967). Where appropriate, the *t* test was used to measure significance of differences, and least significant differences were calculated (Snedecor, 1956).

In most cases, availability of the iron was expressed in terms of relative biological value, to permit comparisons between tests

$$\text{relative biological value} = 100 \times \frac{\text{mg Fe/kg from } FeSO_4}{\text{mg Fe/kg from sample}}$$

to give equal curative effect. Calculations of the actual utilization of iron for the formation of new hemoglobin were made on the assumption that 6.7% of the body weight of the rat was blood, and that hemoglobin contained 3.35 mg iron per g (Greenberg *et al.*, 1957). Various groups, on levels of supplemental iron between 5 and 20 mg per kg of diet, furnished by $FeSO_4 \cdot 7H_2O$ and corrected for the response to iron in the basal diet, utilized from 45 to 51% of the iron supplied for the formation of new hemoglobin.

RESULTS AND DISCUSSION

Comparison of Chicks and Rats as Test Animals. Day-old chicks and weanling rats were chosen as test animals because of their value in previous studies on iron absorption and metabolism, and the possibility that a similarity of response to a specific iron compound in such dissimilar species would favor their acceptability as models of the human in this respect. Accordingly, a series of iron sources were tested with anemic chicks and with anemic rats. The results are summarized in Table I.

While some differences are apparent, agreement between the responses by the two species is generally good. In no instance was a compound poorly utilized by one species and well utilized by the other. Neither species gave a consistently higher relative biological value than the other.

Chickens and rats differ in many respects. In addition to the obvious differences in metabolism, they have widely differing hematological characteristics. Normal values for the chicken are hemoglobin from 7.3 to 12.9 g per 100 ml (average 10.3) and hematocrit from 24 to 43.3% packed cell volume (average 35.6). Normal values for the rat are hemoglobin from 12 to 17.5 g per 100 ml (average 14.8) and hematocrit from 39 to 53% packed cell volume (average 46) (Spector, 1956). Human levels are similar to those for rats (Dacie, 1967).

The day-old chicks depleted much more rapidly than the weanling rats. Severe anemia developed within 2 weeks in the chicks and in 4 to 5 weeks in the rats. This is believed to reflect iron in the maternal diet consumed by the young rats, and not to indicate a species difference.

Preliminary studies, involving increase in plasma iron, following ingestion of test doses by human volunteers, indicate good agreement with the animal feeding results. Expressed as a percentage of the increase that resulted from the same quantity of iron furnished by ferrous sulfate, the following values were observed: ferric orthophosphate 7, ferrous carbonate 4, reduced iron 26, and sodium iron pyrophosphate 7.

Availability of Iron from Dietary Sources. Repletion tests were made on 21 iron compounds and on 14 food sources of iron. The results are summarized in Table II. These include values obtained with chicks and with rats, and include the data shown by species in Table I. Where more than one test was made on a given source, the range of relative biological values is also shown. It should be noted that this range includes both the variation between repeated tests on the same sample and variation between samples when several samples of a given material were studied.

In several cases relative biological values were rounded off to 100, either because the data calculated from hemoglobin and from hematocrit bracketed the 100 figure, or because there were no reference groups in the test that permitted numerical valuation of responses above those obtained with the sample. A typical example is the case of the feed grade ferrous sulfate. This material at a dietary level to furnish 20 mg of iron per kg of diet gave an average hemoglobin value of 10.51 g per 100 ml and an average hematocrit value of 43.9%. The comparable values for the reference reagent grade ferrous sulfate were 10.53 g per 100 ml and 42.7%, respectively.

These observations support the general view that inorganic iron compounds are better utilized than food iron.

Insufficient comparisons were made between similar salts of di- and trivalent iron to confirm the frequently held view that the former are better utilized (Bothwell and Hallberg, 1962; Brown, 1962). Howard (1958) has stated that the rat uses ferric and ferrous salts equally well but that man does not. We found that ferrous chloride and ferrous sulfate were somewhat better utilized by both rats and chicks than the comparable ferric salts.

Among the feed sources of iron, there is no clear distinction in availability between the animal foods and the vegetable foods. This differs from the views expressed by Blanc *et al.* (1968) and Layrisse *et al.* (1968), who considered foods of animal origin to be [illegible] of the iron available.

The iron from [illegible] was well utilized by the rat and chick. This [illegible] to be [illegible] in the human. [illegible] and Smith (1958) found the iron [illegible] to be [illegible].

Table II. Relative Biological Value of Iron from Various Dietary Sources

Iron Source	No. Samples	Relative Biological Value[a] Average	Range[b]
Iron Compounds			
EDTA, dihydrogen ferrous salt	1	99	97-100
Ferric ammonium citrate	1	107	98-145
Ferric choline citrate	1	102	
Ferric chloride	1	44	26-67
Ferric citrate	1	73	70-76
Ferric glycerophosphate	1	93	86-100
Ferric pyrophosphate	1	45	38-52
Ferric orthophosphate	4	14	7-32
Ferric oxide	1	4	0-6
Ferric sulfate	1	83	65-100
Ferrous ammonium sulfate	1	99	99-100
Ferrous carbonate	5	2	0-6
Ferrous chloride	1	98	
Ferrous fumarate	1	95	71-133
Ferrous gluconate	1	97	
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)[c]	1	100	
Ferrous sulfate, anhydrous	1	100	
Ferrous sulfate, feed grade	1	100	
Ferrous tartrate	1	77	70-83
Reduced iron	6	37	8-66
Sodium iron pyrophosphate	3	14	2-23
Food and Feed Ingredients			
Biscuits with ferrous sulfate	1	89	77-100
Blood meal	1	35	
Corn meal enrichment mix[d]	1	46	
Corn germ	1	40	
Egg yolk	1	33	
Fish protein concentrate	2	28	8-53
Enriched breakfast cereal[e]	1	43	
Enriched flour[f]	1	32	
Oat flour	1	21	
Smectite-vermiculite	1	11	3-17
Soybean protein (isolated)	2	97	70-125
Trace mineral mix (commercial)[g]	2	12	0-21
Wheat germ	1	53	

[a] See footnote a, Table I. [b] Lowest and highest values are shown where more than one availability test was made. Note that this reflects variation both between samples and between repeated determinations on the same sample. [c] Fortified with reduced iron. [d] Fortified with ferrous carbonate.

that the iron in eggs is unavailable and that the presence of eggs in the diet interferes with the utilization of iron from other sources (British Ministry of Health, 1968; Elwood, 1968; Elwood *et al.*, 1968; Narula and Wadsworth, 1968).

A biscuit mix was fortified with enough ferrous sulfate to furnish 176 mg of iron per kg, prior to baking. The relative biological value of the iron in the resulting baked biscuits was 77 and 100, respectively, in two tests with chicks. This compared favorably with the arbitrary value of 100 when the ferrous sulfate was added directly to the test diet.

Attention is also directed in Table II to the relative biological values found for the various foods fortified with reduced iron. In all cases these values were within the range found for reduced iron when this material was added separately to the test diet.

Influence of Dietary Protein. In their review articles, Coon (1964) and Layrisse *et al.* (1968) noted that low protein diets interfered with iron uptake. A test was made to compare diets with 10% protein and 20% protein fed to anemic rats. The data are summarized in Table III. With either a well-utilized compound (ferrous sulfate) or a poorly utilized compound (ferrous carbonate), hemoglobin and hematocrit increased numerically when the diet contained the higher protein level. The effect seemed to be greater in the case of the better utilized iron source but the differences were not quite statistically significant.

Table III. Effect of Dietary Protein on Response of Rats to Supplemental Iron

Supplemental Iron Source	mg Fe/kg	Protein %	Hemoglobin (g/100 ml)	Hematocrit (% P.C.V.)
None	0	10	5.40	25.0
Ferrous sulfate	10	10	7.94	37.0
Ferrous sulfate	10	20	8.67	39.7
Ferrous sulfate	20	10	11.36	47.6
Ferrous carbonate	20	10	5.08	25.0
Ferrous carbonate	20	20	5.48	25.2

Table IV. Effect of Ascorbic Acid on Utilization of Iron by Chicks

Iron Supplement Source	mg Fe/kg	Ascorbic Acid (200 mg/kg)	Hemoglobin (g/100 ml)	Hematocrit (% P.C.V.)
None	0		2.82	16.8
Ferrous sulfate	5		4.76	24.3
Ferrous sulfate	10		6.75	28.1
Ferrous sulfate	15		6.96	28.7
Ferrous sulfate	20	–	7.79	30.9
Smectite-vermiculite	20	–	3.05	18.8
Smectite-vermiculite	20	+	3.80	21.7
Ferrous carbonate	20	–	3.29	19.9
Ferrous carbonate	20	+	2.90	18.1
Ferric oxide	20	–	2.78	18.0
Ferric oxide	20	+	3.29	18.6

Influence of Ascorbic Acid and Vitamin E. Many workers have reported that ascorbic acid improves the absorption and utilization of iron (Apte and Venkatachalam, 1965; Banerjee and Chakrabarty, 1965; Brise and Hallberg, 1962; Greenberg *et al.*, 1957; McCurdy and Dern, 1968). Brise and Hallberg (1962) showed that the effect was in the digestive tract, and that intravenous ascorbic acid had no effect. Greenberg *et al.* (1957) reported that the combination of ascorbic acid and vitamin E improved the utilization of dietary iron more than did either vitamin alone. A few laboratories have reported no such effects. Chaney and Barnhart (1960) found that addition of ascorbic acid, sorbitol, and vitamin E did not increase iron absorption by baby pigs. Similarly, in studies with chicks, Hill and Starcher (1968) observed that ascorbic acid had no effect on hemoglobin with or without supplemental iron.

Two chick tests were made to study the influence of ascorbic acid and tocopheryl acetate on the utilization of iron. Table IV summarizes the data on availability of iron from three poorly utilized compounds when 200 mg of ascorbic acid was added per kg of diet. There were no significant differences due to the addition of ascorbic acid to the diet. Table V summarizes results on the relative biological value for ferrous sulfate and ferric orthophosphate in the presence and absence of added ascorbic acid and vitamin E. Ascorbic acid and vitamin E in combination had no significant effect on hemoglobin response. Hemoglobin response was increased by .001% added dl-α-tocopheryl acetate when the basal diet was supplemented with ferrous sulfate, but there was no improvement in response to added tocopheryl acetate when ferric orthophosphate was the iron supplement. Ascorbic acid, or vitamin E, or both together, did not affect the response to added ferric orthophosphate.

Table V. Effect of Dietary Ascorbic Acid and Vitamin E on Utilization of Supplemental Iron

Iron Supplement Added to Diet	Vitamin E (60 mg/kg)	Ascorbic Acid (200 mg/kg)	Relative Biological Value of Iron[a]
None	–	–	[illegible]
Ferrous sulfate	–	–	100
Ferrous sulfate	+	–	97
Ferrous sulfate	–	+	[illegible]
Ferrous sulfate	+	+	125[b]
Ferric orthophosphate	–	–	9
Ferric orthophosphate	+	–	[illegible]
Ferric orthophosphate	–	+	9
Ferric orthophosphate	+	+	10

[a] See footnote a, Table I. [b] Significantly greater than group 2 (ferrous sulfate without added ascorbic acid or vitamin E). Other values do not differ significantly from corresponding control group.

Table VI. Effect of Miscellaneous Foodstuffs on the Utilization of Supplemental Iron

Material Added to Diet or Treatment of Sample Before Mixing into Diet	Relative Biological Value[a] Ferrous Sulfate	Ferric Orthophosphate
Direct addition of iron salt	100	9
$FeSO_4$ mixed with biscuit mix and baked biscuits tested	89	
$FeSO_4$ dissolved in evap. milk	120	
$FeSO_4$ dissolved in skim milk	95	
10% cellulose	92	38
10% lactalbumin	83	
10% soy protein #1	88	
10% soy protein #2	79	
10% gelatin	89	12
5% dried whole egg	100	
15% dried egg white	79	

[a] See footnote a, Table I. [b] Significantly greater than the corresponding control group. Other differences were not significantly different from appropriate control group.

Effect of Miscellaneous Foodstuffs. Brise (1962) reported that food generally interfered with the utilization of supplemental iron given simultaneously. The results of a series of tests in which various foodstuffs were mixed with the iron salt or added to the basal diet are summarized in Table VI. In those cases where a significant quantity of iron was present in test substance, the same quantity of the material was added to the basal diet and to the test diet that contained the iron supplement.

There was no significant difference when ferrous sulfate was (1) added directly to the basal diet, (2) incorporated into a biscuit mix, baked, and then fed in the form of the biscuits, (3) dissolved in evaporated milk before addition to the test diet; or (4) dissolved in skim milk before addition to the test diet. Leichter and Josiyn (1967) reported that when iron bread was found largely in the ferric state regardless of the form in which it is added before baking. The [illegible] show reduced availability.

Dissolving ferrous sulfate in either evaporated or skim milk did not influence its availability to anemic chicks. This agrees with the report by Woodruff (1959) that infants absorb ferrous sulfate added to milk just as efficiently as when ferrous sulfate is given alone.

Addition of high protein foodstuffs to the diet did not have much effect on the availability of iron added in the form of ferrous sulfate. The results were essentially the same when lactalbumin, soy protein, gelatin, dried whole egg, or dried egg white was used. In most cases, the relative biological values tended to be lower in the presence of the high protein

Table VII. Effect of Increased Dietary Levels of Poorly Utilized Sources of Supplemental Iron

Iron Supplement Compound	mg Fe/kg	Hemoglobin g/100 ml	Hematocrit (P.C.V.)
First Test, with Chicks, on Ferrous Carbonate with Relative Biological Value = 0			
None	[illegible]	[illegible]	19.[illegible]
Ferrous [illegible]	[illegible]	[illegible]	2[illegible]
Ferrous sulfate	10	[illegible]	2[illegible]
Ferrous sulfate	15	[illegible]	[illegible]
Ferrous carbonate	15	1.29	18.9
Ferrous carbonate	[illegible]	[illegible]	[illegible]
Second Test, with Chicks, on Reduced Iron with Relative Biological Value = 43			
None	0	2.[illegible]	[illegible].4
Ferrous [illegible]	5	4.[illegible]	22.3
Ferrous sulfate	10	5.[illegible]	25.0
Ferrous sulfate	15	6.5[illegible]	27.5
Ferrous sulfate	2[illegible]	[illegible].[illegible]	2[illegible].0
Reduced iron	2[illegible]	[illegible].[illegible]	[illegible].5
Reduced iron	40	[illegible].[illegible]	28.8
Reduced iron	80	7.32	30.3
Third Test, with Rats, on Ferric Orthophosphate with Relative Biological Value = 15			
None	0	4.98	26.0
Ferrous sulfate	10	7.88	36.4
Ferrous sulfate	20	9.82	44.4
Ferrous sulfate	5[illegible]	13.10	52.0
Ferric phosphate	2[illegible]	[illegible].66	29.6
Ferric phosphate	40	[illegible].82	31.[illegible]
Ferric phosphate	[illegible]	11.[illegible]5	45.2
Fourth Test, with Rats, on Sodium Iron Pyrophosphate with Relative Biological Value = 19			
None	0	4.76	27.0
Ferrous sulfate	10	6.91	36.1
Ferrous sulfate	20	[illegible].[illegible]	42.2
Sodium iron pyrophosphate	2[illegible]	[illegible].67	30.8
Sodium iron pyrophosphate	[illegible]	[illegible].[illegible]	[illegible].2
Sodium iron pyrophosphate	[illegible]	[illegible]	[illegible]

[illegible] Boutrif et al. (1965) have reported that [illegible] with the absorption of food iron. Most of the [illegible] have been shown to be [illegible] agents. Of the [illegible] tested with ferric orphosphate [illegible] had little or no effect on the utilization of [illegible]

[illegible] Effect of Increased Dietary Levels of Poorly Utilized Sources of Supplemental Iron. [illegible]

effective as an oral hematinic for swine even at levels much in excess of the suggested requirement level.

When increased levels of reduced iron, ferric orthophosphate, or sodium iron pyrophosphate were used, these compounds were effective for the cure of iron-deficiency anemia in our experimental animals. The effectiveness was in the order of magnitude that would be expected from the relative biological values established in earlier tests with these materials. These observations indicate that iron sources with at least some minimal availability can be used for food fortification provided enough of the source is used to furnish the needed quantity of available iron. In some applications, technological problems (rancidity; discoloration) may make it impractical to use iron compounds that have maximum availability. These observations provide an alternate mode for food fortification.

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Aqueous Oral Preparations Containing Ascorbic Acid, Vitamin B_{12}, and Ferrous Gluconate*

By C. F. GERBER, C. P. HETZEL, O. KLOZE, and A. F. LEYDEN

Ascorbic acid, vitamin B_{12}, and ferrous gluconate, although mutually incompatible, may be compounded into stable, aqueous oral preparations by using commercial 70 per cent sorbitol solution as a vehicle. Data are presented showing the stabilizing effects of sorbitol in two formulations containing ascorbic acid, vitamin B_{12}, and ferrous gluconate, and in a multivitamin formulation from which ferrous gluconate was excluded. Stability of these products is attributed specifically to their sorbitol vehicles.

Ascorbic acid, vitamin B_{12}, and ferrous iron salts are mutually incompatible. The incompatibility of vitamin B_{12} with ascorbic acid in aqueous solution is extreme. Complete loss of cyanocobalamin and ascorbic acid, for example, was observed in an aqueous solution stored for five weeks at 25°. Similar studies (1-3) of such solutions have emphasized the serious nature of the vitamin B_{12}-ascorbic acid incompatibility, although activity loss was not as marked.

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The incompatibility between ferrous iron salts and vitamin B_{12} is less pronounced. In our studies an aqueous solution (pH 4) of cyanocobalamin and ferrous gluconate lost 2% per cent of its vitamin B_{12} activity after five weeks' storage at 37°, 44 per cent after three months' storage and 96 per cent after nine months' storage at room temperature. Another aqueous solution of ferrous sulfate and cyanocobalamin showed 55 per cent loss of vitamin B_{12} activity after 5 weeks' storage at 37°.

While the destructive action of iron salts on ascorbic acid is generally known, little has been reported concerning the extent of this incompatibility. Ferric iron salt cause more destruction

than ferrous iron salts, but both are extremely deleterious to vitamin C in aqueous solution. A solution of ascorbic acid and ferrous gluconate, adjusted to pH 4.2 and stored for two weeks at 37°, lost 28 per cent of its activity, whereas a corresponding solution which contained an equivalent amount of iron in the form of ferric ammonium citrate lost 61 per cent activity. After four weeks' storage at 37° both solutions showed an 82 per cent loss of activity.

Investigations in our laboratories were directed toward preparing practical aqueous dosage forms containing vitamins B_{12} and C. The effect of ferrous gluconate on stability of the vitamins was also considered.

Initially, the relationship of low water content of such preparations to stability of the vitamins was studied. Multivitamin solutions were prepared using aqueous glycerin (67–70 per cent glycerin) as a vehicle. These formulations were not satisfactorily stable as will be discussed later. Furthermore, liquid formulations containing high concentrations of glycerin are quite unpalatable.

Attention then turned to 70 per cent sorbitol solution as a vehicle for preparations of this type. Commercial sorbitol solution (Sorbo®)[1] has a low water content and possesses a pleasant, sweet taste. It was found that use of 70 per cent sorbitol solution, either alone or with glycerin, as a vehicle for oral liquid products containing ascorbic acid, vitamin B_{12}, and ferrous gluconate circumvents the mutual incompatibilities of this combination.

Two formulations containing this combination and a multivitamin liquid, which does not include ferrous gluconate, are discussed here. The composition of these products is given in Table I.

EXPERIMENTAL

Formulation A (Hematinic). The hematinic preparation was the simplest of the three preparations. It contained ferrous gluconate, vitamin B_{12}[2], and ascorbic acid; 70% sorbitol solution alone was used as the vehicle.

The ferrous gluconate was dissolved in the 70% sorbitol solution at 70°. The temperature was dropped to 45° and the ascorbic acid was dissolved. The solution was cooled to room temperature and the vitamin B_{12} was added. The solution was finally adjusted to pH 4.0 with sodium citrate and flavored.

Formulation B (Multivitamin Drops). The multivitamin drops contained no iron salt because of the high concentration required for adequate iron dosage in 0.6 cc. and because of incompatibility with vitamin A. This dosage form contained vitamin B_{12} and ascorbic acid, with vitamins A and D and B complex vitamins. The vehicle was composed of three volumes of 70% sorbitol solution to one volume of glycerin.

The niacinamide and riboflavin 5′ phosphate sodium were dissolved in 70% sorbitol solution at 45°. This temperature was maintained and the pyridoxine hydrochloride was dissolved. The rest of the vitamins, including vitamin B_{12}, were dissolved in this solution at room temperature.

Vitamin A Palmitate for Aqueous Dispersion (Aquoid®, Pfizer) and vitamin D_2 in corn oil were mixed and diluted further with a small amount of corn oil. This oil solution was then used to form a primary emulsion according to the National Formulary X method for cod liver oil emulsion. One part of powdered acacia (by weight) was dispersed in four parts of oil solution containing vitamins A and D. Two parts of 70% sorbitol solution (by weight) were added quickly and emulsification was accomplished using a Waring Blendor.

TABLE I.

	Concn. / Dose	Concn. / cc.
Formulation A (Hematinic); Dose = 5 cc.		
Vitamin B_{12}, mcg.	2	0.4
Ascorbic acid, mg.	100	20
Ferrous gluconate, mg.	85	17
Vehicle: Sorbo®; flavors; pH, 4.0		
Formulation B (Multivitamin Drops) Dose = 0.6 cc.		
Vitamin B_{12}, mcg.	1	1.6
Ascorbic acid, mg.	50	82.2
Vitamin A, units	5,000	8,333
Vitamin D, units	1,000	1,667
Thiamine hydrochloride, mg.	1	1.6
Riboflavin, mg.	1	1.6
Pyridoxine hydrochloride, mg.	1	1.6
Niacinamide, mg.	10	16.7
Panthenol, mg.	2	3.3
Vehicle: Sorbo®, 75% v/v; glycerin, 25% v/v; acacia, 1 mg./cc.; corn oil, q.s.; flavors; pH, 4.0–4.5		
Formulation C (Tonic); Dose = 10 cc.		
Vitamin B_{12}, mcg.	5	0.5
Ascorbic acid, mg.	100	10
Ferrous gluconate, mg.	50	5
Liver fraction I, mg.	50	5
Folic acid, mg.	0.33	0.33
7-Amino-3-methyl-sulfate, mg.	1	0.1
Methionine, mg.	2	0.2
Panthenol, mg.	2	0.2
Vehicle: Sorbo®, 67% v/v; glycerin, 33% v/v; CMC, 2.5%, (w/v), ethanol, 10%, (v/v), flavors; pH, 4.0–4.2		

[1] Registered trade mark, Atlas Powder Company, Wilmington, Del.

[2] Vitamin B_{12} used in the study was Cyanocobalamin, U.S.P. XV.

[3] Registered trade mark, Atlas Powder Company, Wilmington, Del.

[4] A riboflavin 5′ phosphate sodium preparation from Merck & Co., Inc., Rahway, N.J., was used in this study.

[5] Registered trade mark, Hercules Powder Co., Wilmington, Del. A solution of sodium carboxymethylcellulose in 70 Percent Low in glycerin.

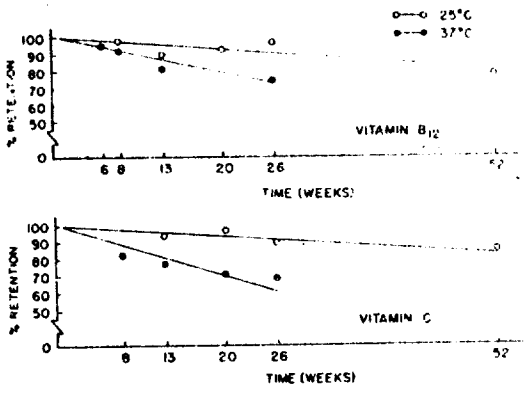

Fig. 1.—Formulation A. Stability of vitamins B_{12} and C at 25° and 37°.

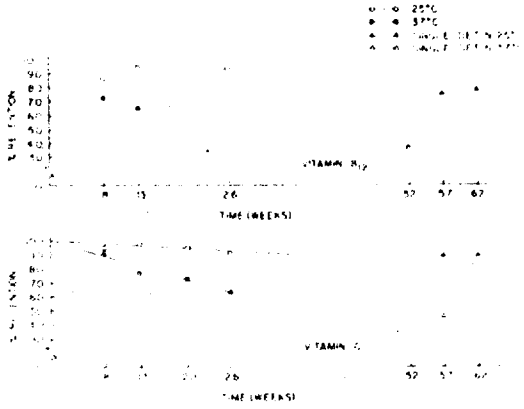

Fig. 2.—Formulation B. Stability of vitamins B_{12} and C at 25° and 37°.

The primary emulsion was then gradually diluted under gentle agitation with the 70% sorbitol solution containing the water-soluble vitamins. The solution was then adjusted to pH 4.0–4.5 with sodium citrate and flavored. The glycerin was added as the final step.

Formulation C (Tonic).—The tonic contained ferrous gluconate, vitamin B_{12}, and ascorbic acid, with other hematinic factors and additional components intended to impart a sense of well-being. The vehicle contained 10% ethanol; the remaining 90% of the vehicle was made up of three volumes of 70% sorbitol solution and one volume of glycerin, which contained 20 mg. sodium carboxymethylcellulose[3] per cc. glycerin.

The ferrous gluconate was dissolved in the 70% sorbitol solution at 70°. The temperature was reduced to 45° and the ascorbic acid was dissolved. After cooling the solution to room temperature, folic acid and thyroxine, dissolved in a minimum volume of 0.1 *N* sodium hydroxide, were added. The steroids, amphetamine sulfate, and vitamin B_{12} were dissolved in the ethanol and dispersed in the main solution. Sodium citrate was used to adjust the preparation to pH 4.0–4.2 and flavors were added.

Finally, the 2% solution of sodium carboxymethylcellulose in glycerin was added. The CMC was included to restore the viscosity which was reduced sharply by the presence of ethanol.

Storage and Assay. In all three formulations principal interest was in the retention of potency by vitamins B_{12} and C which are reputedly unstable when present in the same dosage formulation. In the multivitamin drops (Formulation B) occasional assays were performed on other labile components, notably thiamine and vitamin A.

The samples were subdivided into convenience screw capped bottles with no further precautions. The bottles were stored in ovens at 37° and in an air-conditioned room maintained at 25°.

Generally, assays were performed after storage for 13, 26, and 52 weeks. Some lots were also assayed at 8 and 20 weeks.

Ascorbic acid was assayed according to the method in U. S. Pharmacopeia XV for [illegible]

[3] CMC 70 Premium Low Hercules Powder Company, Wilmington, Del.

with a peroxide modification. Cyanocobalamin was assayed microbiologically using a minor modification of the U. S. P. XV method.

RESULTS

Stability at 25° and 37° of vitamin B_{12} and ascorbic acid in these formulations is shown in Figs. 1–3. Each point on these graphs usually represents an average of assays from nine individual batches of the specified formulation. Straight lines drawn through these points by inspection indicate decomposition trends of the vitamins.

Stability at 25°

Formulation A (Hematinic).—Vitamin B_{12} lost 20% of its initial activity after one year's storage. During this period ascorbic acid lost 19% activity (Fig. 1).

Formulation B (Multivitamin Drops). After six months' storage 8% losses of vitamin B_{12} and ascorbic acid were observed (Fig. 2). Only a single batch of this formulation was assayed after 57 and 62 weeks' storage. The proximity of these points to the extrapolated decomposition trend lines is noteworthy.

Vitamin A also showed an 8% loss after six months. It is interesting that thiamine hydrochloride proved to be the least stable vitamin in this

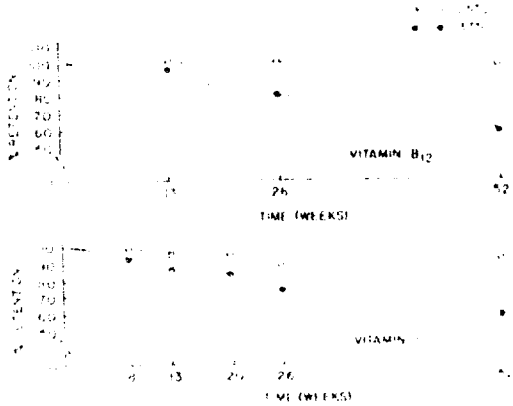

Fig. 3.—Formulation C. Stability of vitamins B_{12} and C at 25° and 37°.

product. There was an average loss of 50% activity after one year's storage.

Formulation C (Tonic).—Losses of 3% for vitamin B_{12} and 14% for ascorbic acid were found after one year's storage (Fig. 3).

Stability at 37°.

Vitamin B_{12} and ascorbic acid decomposition in formulations stored at 37° was similar but progressed at a more rapid rate than in preparations stored at 25° (Figs. 1–3). The data at 37° were not correlated with stability behavior at 25°, and are presented solely for information.

Results of assays on single batches of Formulation B for vitamin B_{12} after 12 months' storage, and for ascorbic acid after 57 weeks' storage are shown in Fig. 2. These results indicate that the slopes of the estimated decomposition trend lines for this product at 37° are inordinately steep.

In our laboratories accelerated aging tests on vitamin preparations have been found useful as a means of determining gross incompatibilities in such formulations, and, when exceptional stability is exhibited at an elevated temperature, as an indication of "room temperature" stability. Tests of this type are also useful in estimating overages in finished dosage forms to compensate for variability of "room temperature" shelf storage conditions. However, an accurate measure of a product's shelf stability is obtained only on samples stored at 25° or at "room temperature."

DISCUSSION

The only component common to Formulations A, B, and C, other than ascorbic acid and vitamin B_{12}, is commercial 70% sorbitol solution. Stability of these formulations is attributed specifically to their sorbitol vehicles. Table II shows overages suggested for maintenance of labeled activity of these products for one year at room temperature, based on their behavior at 25°.

TABLE II.—SUGGESTED OVERAGES[a]

	Vitamin B_{12} Activity Retention[b] %	Vitamin B_{12} Overage, %	Ascorbic Acid Activity Retention[b] %	Ascorbic Acid Overage, %
Formulation A	80	25	81	25
Formulation B	84	20	84	20
Formulation C	97	10	86	20

[a] Sufficient to maintain labeled activity for one year at room temperature.
[b] Activity retention as determined after storage for one year at 25°.
[c] Activity retention estimated for one year at 25°.

As mentioned earlier, glycerin-water systems proved unsuccessful as vehicles for liquids containing ascorbic acid and vitamin B_{12}. Two multivitamin formulations containing 33 and 30% (v/v) water (Formulations D and E) were studied in this connection (Table III).

After 6 months' storage at room temperature, two batches of D lost an average of only 11% of vitamin B_{12}, but 51% of ascorbic acid; after 12 months' storage, these losses had increased to 29% for vitamin B_{12} and 98% for ascorbic acid.

Opposite results were found with two batches of E. After storage for 3 months at room temperature, the average loss of ascorbic acid was only 3%, but there was a 78% loss in vitamin B_{12} activity.

The good stability performance of ascorbic acid and vitamin B_{12} in 70% sorbitol solution may be attributed to two possible causes: the reduced availability of water in sorbitol solution or complex formation between the polyol and either or both of the vitamins.

The specific gravity of Sorbo is about 1.3; thus, 100 cc. contains 39 Gm. water. Since vitamins B_{12} and C showed good stability in Formulations A, B, and C and not in the glycerin preparations (D and E), it follows that the water in the former products may not be as available to cause chemical interaction between the vitamins.

Notably, Formulation A, in which 70% sorbitol solution alone constituted the menstruum, contained more water per unit volume than Formulations D or E (39 cc./100 cc. *vs.* 33 and 30 cc./100 cc.). Thus, it cannot be concluded that the absolute water content of Formulations A, B, and C fully explains the stabilizing effect of sorbitol.

Reduction of reactive water through water-binding by sorbitol could be involved in stability of these formulations. Sorbitol has humectant properties. Solutions of sorbitol lose water more slowly than do glycerin solutions when transferred from high to low humidity conditions (4). This indicates that sorbitol may "bind" water more tightly than glycerin to make it less available for

TABLE III

	Formulation D: Dose = 5 cc. Conc'n/Dose	Amt. Conc'n cc	Formulation E: Dose = 0.6 cc. Conc'n/Dose	Amt. Conc'n cc
Vitamin B_{12}, mcg.	10	2	5	8.3
Ascorbic acid, mg.	50	8	25	41.7
Vitamin A, units	5,000	1,000	5,000	8,333
Vitamin D, units	1,000	200	1,000	1,667
Thiamine hydrochloride, mg.	2.0	0.4	2	3.33
Riboflavin, mg.	1.2	0.24	0.5	0.8
Pyridoxine hydrochloride, mg.	1.0	0.2	1.0	1.67
Niacinamide, mg.	20	4	20	33
Calcium pantothenate, mg.	3	0.6	3	5
Gentisic acid ethanolamide	1.2% (w/v)		0.2% (w/v)	
Sodium benzoate	0.2% (w/v)			
Vehicle[c]				

[a] As riboflavin-5′ phosphate sodium (riboflavin 5′ phosphate ester monosodium salt dihydrate, Pfizer).
[b] Panthenol was used instead of calcium pantothenate.
[c] Formulation D: water, 33% (v/v); Tween 80, 5% (v/v); glycerin, *q.s.*; pH 4.2. Formulation E: water, 30% (v/v); Tween 80, 10% (v/v); glycerin, *q.s.*; pH 4.4.

chemical interaction between the vitamins. The water in starch, for example, tends less to induce or foster a given chemical action than an equivalent amount of free distilled water. A similar phenomenon may be part of the stabilizing effect observed when 70% sorbitol solution is used as a vehicle for ascorbic acid, vitamin B_{12}, and ferrous gluconate, and would explain the apparent anomaly which exists between Formulations A, B, and C and the glycerin preparations, D and E, when only the water content of these products is considered.

The other possible explanation for stability of these formulations may lie in complex formation between sorbitol and either or both ascorbic acid and vitamin B_{12}. It is also conceivable that water-binding and complex formation by sorbitol contribute jointly toward stability of these products.

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Sci. & Cult. 341, 1953

STUDIES ON THE COMPARATIVE BIOLOGICAL AVAILABILITY OF IRON FROM RICE GRAINS FORTIFIED WITH FERROUS SULPHATE, FERRIC CHLORIDE AND FERRIC ORTHOPHOSPHATE

Rice, like other cereal grains, is known to be a poor source of iron so far as haemopoiesis is concerned. It was of practical interest therefore to study whether the rice-eater's diet could be improved in this regard by fortification of rice grains with different iron salts. In order to observe the comparative effects of different sources of iron compound in enriched rice, the following experiments were undertaken.

The rats were made anaemic according to the technique of Elvehjem and Kemmerer[1]. When the rats were 6 weeks old, their haemoglobin level came down to 40–60 per cent. The rats showing haemoglobin level higher than 50 per cent were rejected. The rats (body weights ranging from 20 to 30 grams) were then divided into 8 groups. Each group consisted of six rats. Groups 1 and 8 were kept as negative and positive controls respectively, whereas groups 2, 3, 4, 5, 6 and 7 were supplied with iron-fortified rice diets. The composition of the basal diet used in this experiment, which has to be a low-iron diet, was as follows :—

Basal mixture	%	*Supplements per 100 gm. mg. of basal mixture*	
Rice (washed, dried & powdered)	80	Thiamine hydrochloride (E. Merck & Co.)	1
Casein (low iron)	11	Riboflavine (do.)	2
Steenbock's salt mixture "4" (without iron)	3	Pyridoxine hydrochloride (E. Merck & Co.)	1
Groundnut oil	3	Calcium pantothenate (Roche)	4
		Niacin (Roche)	2
		Choline Chloride (B.D.H.)	100
		Inositol (E. Merck & Co.)	100

Besides these, each rat received a daily dose of 0.03 mg. of copper as copper sulphate and weekly dose of 2 drops of Adexolin (Glaxo) orally.

Rice used in the diet was only of one variety (*viz.*, *Patnai*, parboiled, polished and

of 11.5% moisture content. The iron content of the rice was found to be 35μg. per gm. The fortification of rice grains with different iron compounds was done by suspending the powdered rice grains for half an hour in minimum quantity of water containing requisite amount of different iron salts. The whole suspension was dried at 70° to 80°C. to a final moisture content of approximately 4%. The dried mass was then ground for subsequent use in the diet. Iron salts were added to rice grains at two different levels *viz.*, 35μg. and 55μg. per gm. Low-iron casein, containing approximately 20 μg. of iron per gm., was prepared from skimmed milk by precipitation with N/10 HCl, dried at 60°C. and finally defatted by ether extraction.

In order to maintain the same average food consumption, all the groups were paired-fed in respect to the diet taken by the negative control group. The experimental feeding period lasted for 4 weeks. Haemoglobin determinations and weighings were made at the end of each week. Haemoglobin was determined by Sahli's haemoglobinometer in 0.02 cc. of blood taken from the tail of the rat.

The results of haemoglobin determinations at different stages revealed that after 28 days, at an iron intake level of 35μg. per gm. of rice, the corresponding haemoglobin values of Gp. 2. ($FeCl_3$-supplemented), Gp. 4 ($FeSO_4$-supplemented), Gp. 6 ($FePO_4$-supplemented) had increased by 47, 43 and 20 units respectively, whereas at the iron intake level of 55μg. per gm. of rice, the haemoglobin levels of Gp. 3 ($FeCl_3$-supplemented), Gp. 5. ($FeSO_4$-supplemented) and Gp. 7 ($FePO_4$-supplemented) had increased by 48, 47 and 33 units respectively. The corresponding haemoglobin value of Gp. 1 (negative control) was found to increase by 14 units only. In four weeks, the haemoglobin values of different groups supplied with iron-enriched rice diet had thus increased to a considerable extent in comparison to the negative control groups where no iron fortified rice diet was given. Comparing the figures for haemopoiesis at two different levels of iron intake, it appeared that ferric chloride and ferrous sulphate had greater haemopoietic effect than ferric ortho-phosphate in enriched rice grain. Between ferrous sulphate and ferric chloride, there was no appreciable difference so far as haemoglobin regeneration was concerned.

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J. J. Ghosh

Nutrition Research Unit,
Department of Applied Chemistry,
University College of Science & Technology, Calcutta.
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Ferrous Sulfate Poisoning

DAVID M. GIMLETT, M.D. San Jose, California

Although many articles have appeared in the medical and lay literature concerning accidental poisoning of children with medicinal iron, recent increase in the incidence of this problem appearing at our hospital for treatment has pointed out too clearly that this hazard of iron therapy needs further publication. Furthermore, the surprise expressed by many physicians when the cases below were brought to their attention is evidence that the public alone is not responsible for lack of attention to this hazard. The purpose of this article is to re-emphasize the problem and to review the mechanisms and treatment of iron poisoning. Importance of this problem is not limited to the pediatricians who may be treating the victims of iron ingestion, but it applies to the general practitioners, internists, surgeons, gynecologists and obstetricians who are responsible for the inclusion of iron salts on the medicine shelves of their patients. This problem is especially pertinent to this latter group because it is their patients who are most likely to have young children in the household.

A further purpose of this article is to emphasize an important aspect of the clinical picture of iron poisoning, the neglect of which contributed to the death reported below and attention to which may prevent similar tragedies in the future. This aspect is the period of apparent well-being described below as phase two of the clinical picture.

At our hospital from the period January, 1950 through December, 1960, there were two cases of accidental iron poisoning with no fatalities. On the other hand, from September 28, 1961, to April 1962, there were nine cases, one of which was fatal. In most series the mortality rate of this accident is about 50 per cent. The low mortality rate in our series may be due to the fact that the diagnosis was made on the basis of history alone and that definite proof of iron ingestion was not established in every case.

From Mountain View General Hospital, Tacoma.

Fouks, in 1947 was the first person to report on medicinal iron poisoning. Since that time many reports have appeared and in 1958, R. A. Alfheim reviewed 12 cases from the medical literature. In studying these cases it is important to note that five grams of ferrous sulfate can be fatal to a two year old child.

CASE REPORTS

Case 1. This two year old Indian male had been in good health except for acute tracheobronchitis at the age of 15 months. He ingested what was estimated to be 15 ferrous sulfate tablets, 300 mg., during the morning of September 28, 1961 while he was in the care of a baby-sitter. Nothing was done at this time but when the mother returned to the house at 5 p.m. and was informed of the incident, she brought the patient to the emergency room of this hospital. At that time the child was asymptomatic and physical examination revealed no abnormalities. Bowel sounds were normal and stool guaiac was negative. The patient was lavaged with 1500 cc. of water but no pills were recovered. He was given one ounce of castor oil and sent home. The mother was told to return if there was emesis or diarrhea.

During the night the child began to vomit and did so intermittently during the rest of the night and the next morning. He was brought to the emergency room at 11:45 a.m. in stuporous condition with temperature of 104.2 F. He was taken to the pediatric floor at which time his blood pressure was unobtainable and he had Cheyne-Stokes respirations. He was markedly cyanotic, comatose, and his lungs were dry but wet rales. He was placed in an oxygen tent and a venous cut-down was performed. Infusion of five per cent dextrose and saline was started but respirations ceased and resuscitation methods were without effect. The child was pronounced dead at 1:10 p.m., about 27 hours after ingestion of the iron.

Pertinent pathological findings were limited to the small intestine and spleen. The stomach and duodenum were normal, with intact mucosa. The jejunum, ileum and colon were dilated to 3 cm. in diameter, the dilation extending to about 12 inches from the ileocecal valve where the bowel narrowed to 1.5 cm. At 6 inches from the ileocecal valve and extending upward 6 inches, the serosa was covered by a thick purulent exudate and the bowel was gangrenous. Discoloration to a lesser degree extended proximally along the ileum and through about 1/3 of the jejunum. Bowel content was a greenish purulent liquid. At the site of the peritonitis the bowel was dilated 2.5 thin discs about five mm. in diameter which were consistent with tablets of ferrous sulfate.

Figure 1 Case 1. X25. Small bowel wall stained with Gomori's Iron Reaction. Hemorrhagic necrosis of the bowel is seen with iron extending out to the peritoneal surface

None were found beyond this particular area. It would appear that the pills had caused obstruction at this point. Microscopic section of the small bowel showed hemorrhagic necrosis of the tissue with marked vascular congestion and infiltrate of polys. Iron stain showed a marked amount of iron in the focal contents and also extending to a minor degree out into the wall and onto the peritoneal surface. See figure 1. There was an unusual type of giant cell in the follicles of the spleen. This was a multinucleated histiocyte in the germinal centers of the spleen without any other change in the tissue that might account for its presence.

Case 2. This 17 month old girl ingested an unknown number of candy-coated 300 mg. ferrous sulfate tablets while her mother was sleeping, about two and a half hours before she was brought to the emergency room of our hospital. She had vomited four times during that period. She was lavaged with one liter of water and given one ounce of castor oil and admitted to the hospital. When she arrived on the floor she was a sleepy, limp child who could be aroused but would fall right back to sleep. Her systolic blood pressure was 60 by flush technique and she had peri-orbital and perioral cyanosis. Her breathing was somewhat shallow but otherwise there were no abnormalities. Intravenous fluids were started but they infiltrated. A cut-down was then performed. The child was also given 200 cc. of 5 per cent sodium bicarbonate solution by mouth. After 350 cc. of Polyral had been given intravenously, she became more alert, her blood pressure rose to 80 systolic, and the cyanosis disappeared. With this treatment, her course was one of steady improvement, plasma substitutes and exchange transfusion were considered unnecessary. The patient was discharged, apparently well, 48 hours after admission.

Case 3. This three year old white male was admitted to our hospital at 4 p.m., several hours after ingesting approximately 80 tablets containing a total of 24 Gm. ferrous sulfate. He had become lethargic, had started vomiting and had been passing loose stools. When brought to the emergency room he was found to be lethargic and pale. He was passing loose, soft, grey-black stools. His pulse was 120, temperature 98 F and his blood pressure could not be read audibly, but was weakly palpable at 120.

An x-ray was taken which showed 50-60 radiopaque pills scattered throughout the gastro-intestinal tract, including 27 still in the stomach (Fig. 2) in spite of the fact that he had been vomiting. Blood was drawn for iron levels, electrolytes, and typing and cross-matching, and a cutdown was performed. Gastric lavage was attempted but was unsuccessful because the pills were too large to pass through the tube. An intensive effort was then made to rid the patient's gastro-intestinal tract of the pills. Repeated 500 cc. cocktails of ipecac, sodium bicarbonate, milk of magnesia and castor oil were fed to the patient through a catheter straw. After each cocktail the patient would vomit and a few pills would be recovered. The progress of this therapy was followed by x-ray. At ten p.m., over 12 hours after ingestion, four pills could still be demonstrated in the stomach (Fig. 3) so the child was induced to vomit one more time and these were recovered. One of these pills was almost intact except for the loss of its enteric coating.

The initial laboratory data showed the electrolytes to be essentially normal and the serum iron to be 326 mcg. per 100 ml. Stools were positive for occult blood.

Six hours after admission the blood pressure could be read audibly and the pulse was stronger. The serum iron was still 315 mcg. per 100 ml. Exchange transfusion was contemplated but by 14 hours after admission the patient was more alert and the serum iron level was down to 73 mcg. per 100 ml. (the child was originally being treated for milk anemia). He went on to complete recovery 48 hours after ingestion. In this case it was not found necessary to give vasopressors, intensive electrolyte therapy, or exchange transfusion.

clinical picture

The cases described above illustrate many of the important features of the clinical picture of iron poisoning. Aldrich, after review of 42 cases, outlined four phases in the iron toxicity syndrome. The first phase occurs within 30 to 40 minutes after ingestion. It is characterized by vomiting (which may produce brown or bloody emesis), diarrhea, and abdominal pain or discomfort. The child may become irritable, pale, and drowsy, pulse may become weak and Kussmaul respirations may appear. Another characteristic of this phase not mentioned by Aldrich but which we observed in one of our cases and Ammann[5] reported in his, was peri-oral and peri-orbital cyanosis. During this phase increased

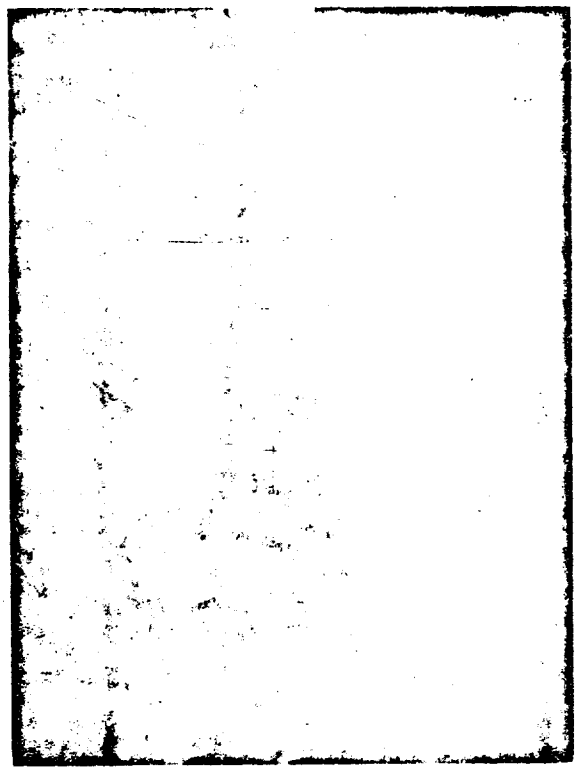

Figure 2. Case 3. Admission x-ray of the abdomen demonstrating 50-60 radiopaque pills scattered throughout the gastro-intestinal tract.

signs of cardio-vascular collapse may develop and death may occur in less than six hours, as it does in about 42 per cent of the patients who die of iron poisoning, or the patient may progress into phase two.

This second phase is characterized by return of color, pulse, respiration, and level of consciousness to normal, with decrease or even cessation of the nausea, vomiting and diarrhea. It is during this phase that the physician often first sees the child and if he is unaware of this misleading aspect of the iron toxicity syndrome, he may well assume, as was done in the first case presented above, that the child has expelled all of the ingested iron and the the hazard to his life has passed. The possible dire consequences of such an assumption are well illustrated above. This second phase may lead to improvement and complete recovery, or, at the end of 10 to 14 hours, it may lead into the third phase which is characterized by cardiovascular collapse, Cheyne-Stokes respiration, convulsions, and coma, until death finally occurs any time from 20 to 50 hours after ingestion. Fifty per cent of the deaths occur in this period.

The last phase occurs one to two months after the initial insult and is caused by the cicatricial changes which can result from the direct necrotizing effect of the iron compound on the gastrointestinal system. Pyloric or lower intestinal obstruction has necessitated surgical intervention and, when neglected, has even led to death by malnutrition.

As in any syndrome, individual cases may deviate from the typical pattern. Our first case is a good example of this in that the first and second phases seem to have been inverted. The crucial point is, however, that here, too, there was a deceptive period of apparent well-being which biased the manner in which the case was handled.

pathologic picture

Necrotizing gastritis and enteritis is a common occurrence in iron poisoning but not a necessary part of it. If the tablets are enteric coated and have not been chewed, the stomach will not be affected. When gastritis does occur, the mucosa is found to be congested with sludging of blood in the capillaries. Ferrous and ferric iron are

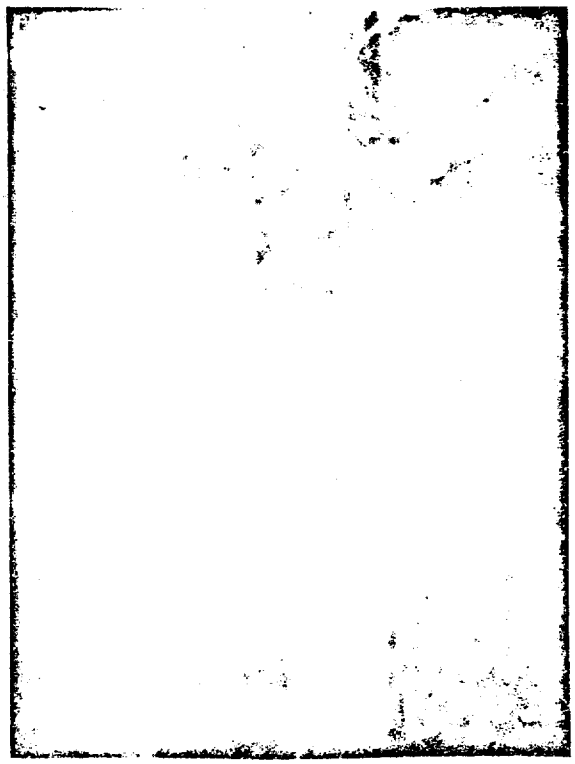

Figure 4. Case 3. X-ray taken more than twelve hours after ingestion of 50-60 iron tablets. Four pills can still be seen in the stomach.

found in the mucosa, connective tissues, basement membrane, and endothelial lining of the vessels. Platelet thrombi may be found within the vessels. The liver may show peri-portal necrosis with deposits of iron in its reticuloendothelial elements.

The lungs, brain, kidneys and heart show edema, cloudy swelling and areas of hemorrhage. Reissmann[1] showed that hemorrhagic changes occurred in the lungs even when aspiration was impossible such as with rectal administration of the iron. The pulmonary edema which occurs can often be detected clinically and is important in the mechanism of death. In Reissmann's animals, respiratory failure was the direct cause of death in most cases.

mechanism of action

A brief review of iron metabolism might be helpful in elucidating the mechanism of toxicity of ingested iron and aid in an understanding of the measures which are advocated for the treatment of this emergency.

The total iron in the adult body is four to five grams, more than 80 per cent of which is in combination with porphyrins as heme compounds, myoglobin, and respiratory enzymes. About 20 per cent of it exists as storage iron, primarily ferritin and about 0.1 per cent as plasma iron, chiefly transferrin. Storage iron consists of ferritin and hemosiderin. The former is a combination of ferric hydroxide-phosphate and a protein, apoferritin. This protein is apparently identical to the vasodepressor material (VDM) described in 1948 by Shorr et al[2] and its production is stimulated by the presence of ferritin. It has been postulated by Aldrich that [illegible] may be responsible for [illegible] lapse seen in the iron toxic[illegible]

Plasma iron is [illegible] trace of it is free iron. Most [illegible] combination with beta-[illegible] called siderophilin. [illegible] globulin is nearly [illegible] only one-third saturated [illegible] iron concentration [illegible] 100 ml.

gastric absorption

It has been stated [illegible] absorbed from the [illegible] digestion of iron, but [illegible] in most of the cases of iron poisoning [illegible] responsible for [illegible] able iron being absorbed through the gastric mucosa. Heilmeyer demonstrated that a high degree of gastric absorption of iron occurred in animals after ligation of the pylorus and separation of the duodenum.[3] According to him, "Practically speaking, without the help of the small intestine, the stomach alone can absorb all the iron administered."

Gastric acidity aids in the absorption of iron by keeping it from being precipitated as the phosphate. Protein hydrolysis also increases the solubility of iron by preventing the production of iron proteinates. Iron is absorbed through the mucosal cells as ferrous iron and then immediately oxidized to the ferric state, combined with apoferritin, and stored as ferritin.

mucosal block?

The theory of *mucosal block* states that in anemia the oxygen content of the mucosal cells is reduced, and some of the mucosal iron is reduced to the ferrous form. It is then carried away into the blood stream to the bone marrow. The reduction in mucosal ferritin allows more iron to be absorbed until the anemia is corrected and the oxygen content of the mucosal cells is returned to normal.

It is obvious that large doses of ingested iron are able to overcome this mucosal block. Heilmeyer, however, concluded that there is no such thing as a mucosal block since, under his experimental conditions, liver iron continued to [illegible] when mucosal ferritin levels were maximal and no absorption of iron took place when the mucosal ferritin level was declining. Kiedertag and Weber, using radioactive iron absorption studies, demonstrated that iron absorption [illegible] time of maximal ferritin concentration in the duodenum.[4] Cann and Verhulst state that in poisoning the small bowel mucosa is damaged allowing excessive iron absorption. [illegible] dogs, reproduced the clinical picture of iron poisoning in children and [illegible] show that high serum levels of non-[illegible] iron could be [illegible] within 60 minutes in the absence of [illegible] Hyperventilation was present within one hour, secondary to the [illegible] acidosis which in turn [illegible] to be due to conversion of ferrous iron to the ferric form and the production [illegible]

[illegible] compound is unstable and [illegible]

complexes giving up hydrogen ions in the process. These workers also found hemoconcentration due to a shift of fluid from the intravascular to the extravascular compartment. Furthermore, they demonstrated decreased stroke volume, cardiac output, and blood pressure, and increased pulse rate secondary to decreased venous return.

vascular collapse

It is the vascular collapse which is the lethal factor in iron toxicity and even though intestinal necrosis and peritonitis frequently occur in those cases which come to autopsy, death can also occur in their absence. In our first case reported above, though there was an area of hemorrhagic necrosis in the cecum and local peritonitis, there was no perforation or generalized peritonitis of sufficient extent to be considered the cause of death. Reissmann demonstrated that death could occur without gastro-intestinal changes and concluded that, "the intestinal or gastric necrosis is not a necessary factor or integral part of the fatal outcome of iron poisoning and that the lethal effect is primarily due to the absorbed iron."

treatment

The following procedure is offered as a general plan of management in treatment of this emergency condition. Alterations in the plan will occur with individual cases, of course.

A. Treat the Shock. In the presence of shock, intravenous therapy, through a cutdown, should be started immediately. While blood is being typed and cross-matched (enough sample being drawn for a determination of serum electrolytes and iron), plasma or plasma substitutes may be started. Whole blood can then be administered and the advisability of doing an exchange transfusion may be considered. Vasopressors may also be of some help since the vasodepressor action of VDM probably contributes to the shock syndrome.

B. Empty the Stomach. Induction of emesis should be the first phase of this step since the large iron tablets are not likely to pass through a lavage tube. Following this, the stomach should be lavaged with 5 per cent sodium bicarbonate solution and 20 cc. of the solution should be left in the stomach at the end of the procedure in order to convert what iron remains into the less soluble salt, ferrous carbonate.

If there is no doubt that iron was ingested, this step should be performed as soon as the child is seen and the blood pressure is known to be stabilized since, as has been noted, gastric absorption of iron is possible and toxic symptoms can occur within 30 to 60 minutes of ingestion.

C. X-ray of the Abdomen. This simple and often ignored procedure can be used before the induction of emesis is attempted if there is any doubt that iron was really ingested. If no iron pills show up in the stomach on x-ray, gastric lavage will be pointless and the child can be spared the trauma and potential danger of this procedure. If emesis has been induced, an x-ray of the abdomen will demonstrate if all the pills have been removed. If a number of pills remain in one location, as in our first case, surgical removal may be considered if the patient's condition permits. As can be seen in our third case, serial x-rays of the abdomen are extremely useful in directing the course of treatment. In this case it should be noted that more than twelve hours after ingestion, 1.2 grams of ferrous sulfate could still be detected in the stomach and the pills were successfully removed by further induced emesis. Without the use of x-ray the presence of these pills would not have been suspected this long after ingestion. Demonstration of the retention of these pills after repeated emesis over a prolonged period teaches an important lesson about the treatment of poisonings in general.

D. Empty the Bowel. Cathartics should be given, combined with enemas if necessary, to empty the bowel. Demulcents such as milk, oil, or egg whites may be used to prevent absorption of the iron as it passes through the gut. Bicarbonate or phosphate ions can be provided to decrease the solubility of the iron. Very rapid transit times through the intestine can be achieved so that nearly intact iron tablets can be retrieved in the stool.

E. Exchange Transfusion. This is probably the most efficient method for removal of the absorbed iron from the blood stream and body tissues. If a cutdown has already been performed for the administration of fluids, as proposed above, exchange transfusion can be started with little loss of time if the patient's condition does not respond to the above measures. Amerman et al report on the efficacy of this form of treatment in the case of an 18 month old child who ingested 45 to 75 gr. of ferrous sulfate, vomited and had black stools. Their patient was admitted with peri oral cyanosis, blood pressure of 50 0,

and in semi-comatose condition (much like case 2 above). Six and one-half hours after admission, exchange transfusion was started and the patient subsequently recovered. Whether the transfusion was really necessary is not definitely shown but it seemed to be of use when other measures failed to eliminate the cyanosis.

In view of the problems in blood coagulation which develop as a result of iron poisoning, as elaborated by Wilson et al,[5] this aspect of the treatment program assumes even greater importance and should be emphasized more than it has been in recent articles.[12]

F. Chelating Agents. The use of these agents has been advocated and carried out by a number of people on a theoretical basis but the opinion of the investigators who have examined their use in removing iron in iron storage diseases is practical. It is well summarized in the following statement and probably can be applied equally as well to acute iron poisoning, viz., "In evaluating the use of chelating agents it is apparent that the increases in iron excretion are only a small fraction of the amount removed by a single phlebotomy and it is doubtful that this form of chemitherapy can compete with bleeding as a means of mobilizing iron in primary hemochromatosis."[13] Benson and Sisson used EDTA (ethylenediaminetetraacetic acid) in the treatment of experimental iron poisoning in dogs and were able to lower the serum iron but their animals subsequently died.[13]

Cann and Verhulst correctly state that "the removal of circulating iron from the body by dimercaprol (BAL) has proved disappointing. The dimercaprol-iron complex seems to be more toxic than iron salts."[3]

G. Correction of Acidosis. As mentioned earlier, metabolic acidosis is an early occurrence in the iron toxicity syndrome. When the more important measures have been carried out, the adjustment of electrolyte balance should be performed with M/6 sodium lactate or sodium bicarbonate.

H. Artificial Kidney. The use of this machine has been advocated in the treatment of iron poisoning but it is doubtful if it would add anything to the therapeutic regimen. As Reissmann pointed out, most of the iron in the plasma is in a non-dialyzable form. Precious time could be lost in initiating artificial dialysis when exchange transfusion could be quickly, and probably more effectively, instituted.

I. Hospitalization. Due to the deceptive clinical course of this syndrome, we believe in the hospitalization, immediately after ingestion, of all children in whom there is any doubt that all of the ingested material was removed from the stomach. They should be observed for at least 48 hours and attending nurses and physicians should be instructed not to be deceived by apparent well-being of the patient.

prevention

Of course, the first line of defense in the prevention of accidental iron poisoning consists of the general precautions against any kind of medicinal poisoning in children, viz., keeping medicine containers tightly sealed, out of reach, and locked up.

Another important preventive measure is the proper instruction of the public in the high toxicity of iron salts by labelling the bottles as poisonous to children. As an extension of this it is important for patients and physicians to be aware

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of the various vitamin and hematinic agents which contain iron in significant quantities.

summary

The recent high incidence of iron poisoning with one fatality at our hospital has been illustrated. A number of important points concerning iron poisoning have been emphasized, viz.:

A. The high mortality rate, often running to 50 per cent.

B. The deceptive clinical picture with a period of apparent well-being occurring just prior to the rapid onset of cardiovascular collapse.

C. The significance of this problem to physicians who are prescribing iron compounds to [illegible] patients.

D. The use of x-ray films to demonstrate the presence of iron tablets in the gastro-intestinal system.

E. The important role gastric absorption plays in the production of this syndrome.

F. The advisability of hospitalization in the management of virtually all of these cases. ■

Santa Clara County Hospital

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THE EFFECT PRODUCED ON THE BLOOD FORMATION AND METABOLISM BY FEEDING ACTIVE IRON OXIDE AND RADIOTHORIUM TO NORMAL RABBITS IN CONSIDERATION OF THE URINE QUOTIENT C:N

by Allen Goldbloom (New York)

(from the experimental Biology Department of the Pathology Institute of the University of Berlin)

(received November 10, 1927)

A few years ago at the request of Prof. Bickel Dr. Brodski from Rostow carried out experiments in the local laboratory on normal rabbits for the purpose of determining to what extent it would be possible to bring about an increase in the number of red blood corpuscles by feeding so-called active iron oxide in the sense of Baudisch, now known under the name Siderac. For purposes of comparison normal rabbits were also fed inactive iron oxide according to Baudisch. It turned out that a hyperglobulia was not produced in these normal animals through these iron feedings. The tests of Brodski, which have not been published up to now, were handed over to me by Prof. Bickel for publication in this article. In the table which follows I present the test results achieved by Brodski. All of the rabbits were fed the same carrot feed during the entire test. Every rabbit was then observed for a period of several days with the feeding of iron. Then the rabbits were fed perorally increasing doses of active and inactive iron oxide in immediately consecutive intervals. In the tests with the active iron oxide at the end of them a period was added on lasting several days in which this iron oxide was applied subcutaneously in aqueous solution.

The red blood corpuscles were counted in each test period. In table I the average values of the tests by Brodski (1) are given.

From these figures it follows that we cannot speak of any blood-picture promoting effect of the active and inactive iron oxide in normally grown rabbits which were fed normal food.

It now became conceivable that by means of a combination of active iron oxide and radioactive substance a hyperglobulia could be produced. It was shown by the experiments carried out by Wada (2) in our laboraty that by means of daily feeding of 30 to 44 Mache units (abbreviated M. E.) of radium bromide per kilogram of body weight in normally fed normal dogs a slight hyperglobulia gradually formed. After intravenous single injections of about 50 to 500 M. E. of radium bromide in normal and normally fed rabbits there

occurred a certain tendency towards increase of the red blood corpuscles without any effect on the body weight. By means of the work done by Kosokabe (3) from the local laboratory it was also known concerning radiothorium that with a single intravenous injection of a low dose of about 100 M. E. per kilogram in rabbits there gradually occurred a slight increase in the number of red blood corpuscles . Nothing was known as to the effect of such low radiothorium doses on the blood picture (blood count) in peroral daily repeated doses. Such an effect was however possible when we recall Wada's tests with radium feed. Combination tests of two kinds therefore come into consideration: 1. active iron oxide combined with small doses (about 75 to 100 M. E.) radiothorium and 2. active iron oxide combined with small doses (about 30 to 50 M. E.) of radium bromide whereby these doses were calculated per day and kilogram of body weight. The purpose of these combination tests was , as already stated, that of determining whether by means of this combination of active iron oxide and radio active substances stronger effects could be exerted on the blood-forming mechanism, along with the general metabolism effects, which are inherent to the active iron and the radium or the radio thorium in the dose mentioned.

This present work of mine is concerned with the effect of the feeding of active iron oxide and radiothorium on the formation of blood and the general metabolism in peroral administration of these substances.

In an earlier paper (4) I showed that radiothorium at a dosage of about 75 M. E. per kilogram and per day in daily repeated administrations causes an increase in the urine quotient C:N. The same occurs, as indicated by Wada (5)also in the peroral feeding of active iron oxide. Thus in the low dosage mentioned the radiothorium works the same as the active iron oxide Siderac of which 5 mg per kilogram and per day is an effective dose.

The C in the urine was determined in accordance with the method of Gomez given in his work (reference 7) . The N in the urine was determined according to Kjeldahl in the 24-hour urine amount.

The result of my tests, which is compiled in Table II, was therefore the following:

By means of daily peroral feeding of 5 mg of active iron oxide (Siderac) and 75 M. E. of radiothorium in the case of normal and normally fed rabbits the urine quotient C:N is driven upwards , but an increase in the red blood cells does not take place. It follows from this that the dose of the radiothorium which is optimal in peroral feeding of it for producing the general metabolism effect which is expressed in the increase in the urine quotient is not sufficient to produce, and let this be emphasized, in normal and normally fed animals any change in the blood picture even within several weeks. How the anemic body behaves with regard to such

treatment must be investigated in particular and also the question of whether other results can be obtained in normal and anemic bodies using radiobromide instead of radiothorium. On the other hand, in the peroral feeding of about 150 M. E. of radiothorium and 5 mg of active iron oxide (Siderac) per kilogram and per day , as well as in somewhat greater doses, an increase in the number of red blood corpuscles was achieved. in normal and normally fed rabbits. The dose of 150 M. E. is not as favorable, however, as the dose of 75 M. E. for the effect of the radiothorium as regards increasing the C:N quotient.

Lastly let it also be pointed out that the active iron oxide "Siderac", which we heard about in the beginning, that it does not affect the blood formation in normally fed rabbits, in the case of anemic children the anemia in such children was promptly healed . according to the observations of Moldawski (6).

We must always bear in mind that in the radioactive elements and particularly in the substances of the thorium series it is quite a different matter if we administer a given dose perorally or the same dose intravenously, since in the case of the peroral feeding because of difficulties in reabsorption the amount of substance which is actually fed to the body environment cannot be evaluated at all.

Table 1

1= iron dose per animal and day
2= observation time in days
3= number of red blood corpuscles as an average over the period
4= rabbit No. 1
5= active iron per os (perorally)
6= active iron subcutaneously
7= rabbit 2
8= active iron per os
9= subcutaneously
10= rabbit 3
11= inactive iron per os
12= active iron per os
13= rabbit 4
14= inactive iron per os
15= active iron per os

Table II

1= iron and thorium dose per kil of body weight and per day administerd perorally (through the mouth)
2= observation periods in days
3= the number of red blood corpuscles as an average over the period
4= C:N (quotient) as a period average
5= rabbit (no.) 1
6= 75 Mache units radiothorium + 5 mg active iron oxide
7= rabbit 2 75 Mache units , radiothorium + 5 mg active iron oxide
8= rabbit 3 75 Mache units radiothorium + 5 mg active iron oxide
9= rabbit 4 75 Mache units radiothorium + 5 mg active iron oxide
150 Mache units radiothorium + 5 mg active iron oxide
10= rabbit 5 75 Mache units radiothorium + 5 mg active iron oxide
150 Mache units radiothorium + 5 mg active iron oxide
11= rabbit 6 75 Mache units radiothorium + 5 mg active iron oxide
150 Mache units radiothorium + 5 mg active iron oxide

Table III
Test by Brodski on rabbit no. 1; carrot feed

1= date
2= weight in grams
3= hemoglobin in %
4= number of red blood corpuscles given in millions
5= white blood count
6= from May 25th to to June 1. Without iron
7= from June 2 to June 10. 0.005 g of active iron daily.
8= from June 11 to June 16. 0.03 g active iron oxide daily.
9= from June 17 to June 24. 0.015 g active iron oxide subcutaneously.

Table IV

Test by Brodski on rabbit no. 2; carrot feed

1= date
2= weight in %
3 = hemoglobin in %
4= red blood corpuscle count in millions
5= white blood count
6= from May 25 to Mane 1: pre-period. Without iron.
7= from June 2 to June 11 0.005 g active iron oxide daily.
8= from June 11 to June 16: 0.03 g active iron oxide daily.
9= from June 17 to June 24: 0.05 g active iron oxide subcutaneously.

Table V

1 = Test by Brodski on rabbit no. 3 ; carrot feed

2= date
3= weight in grams
4= hemoglobin in %
5= red blood corpuscles expressed in millions
6= white blood count
7= remarks
8= without iron oxide
9= 0.005 g inactive iron oxide daily per os
10= 0.03 g inactive iron oxide daily per os
11= 0.015 g active iron oxide daily per os

Table VI

1 = Test by Brodski on rabbit no. 4; carrot feed
(same column captions as above in Table V)

2= without iron oxide
3= 0.005 g inactive iron oxide daily per os
4= 0.03 g inactive iron oxide daily per os
5 = 0.015 g active iron oxide daily per os

Table VII

1= Test by illegible on rabbit no. 1 of table illegible

2= date
3= body weight in grams 4= urine amount in ccm
5= urine C in grams 6= urine N in grams
7= C:N quotient 8= urine C in grams 9= urine N in grams
10= carrot quantity consumed in grams per day
11= per day as an average over the period (under columns 8,9,10)
12= Remarks 13= pre-period
14= Dose of 75 M. E. radiothorium + 5 mg active iron oxide per kilogram and per day from August6 to Sept. 8, inclusive.

1 = Table VII a (continuation)

2= date 3= body weight in grams 4= urine amount in ccm
5= urine C in grams 6= urine N in grams 7= C:N
8= urine C in grams 9= urine N in grams 10= C:N
11= per day as an average over the period
12= amount of carrots consumed in grams per day
13= remarks
14= Dose of 75 75 M. E. radiothorium + 5 mg active iron oxide per kilogram and per day from August 6 to Sept. 8, inclusive.

1 = Table VII a (continuation

2= date 3= body weight in g 4= amount of urine in ccm
5= urine C in grams 6= urine N in grams 7= C:N
8= urine C in grams 9= urine N in grams 10= C:N
11 = per day in the average over the period
12= amount of carrots consumed per day in grams
13= remarks
14= after-period
p

1 = Table VIIa. (continuation)
In average over the periods

2= time period
3= body weight in grams
4= amount of food consumed in grams
5= C 6= N 7= C:N 8= remarks
9= iron and radiothorium

1= Table VIIb
Blood count in rabit no. 1 of table II

2= date 3= body weight in grams 4= hemoglobin in %
5= number of red blood corpuscles in millions
6= color index 7= white blood count
8= remarks
9= pre-period- no feeding of iron
10= Dose of 75 M. E. radiothorium + 5 mg active iron oxide per kilogram and per day
11= after (post) period
12 = as an average over the periods
13= pre-period
14= main period
15 = after-period

1 = Table VIIIa. Test by Goldbloom
on rabbit no. 2 of table II. Carrot feed.

2= date 3= body weight in grams 4= amount of urine in ccm
5= urine C in grams 6= urine N in grams 7= C:N
8= urine C in grams 9= urine N in grams 10= C:N
11= per day as an average over the periods
12= amount of carrots consumed per day in grams
13= remarks
14= pre-period 15= Dose of 75 M. E. Radiothorium + 5 mg active iron oxide per kilogram from July 11 to July 13.

1 = Table VIIIa (continuation)

2= date 3= body weight in grams 4= amount of urine in ccm
5= urine C in grams 6= urine N in grams 7= C:N
8= urine C in grams 9= urine N in grams 10= C:N
11 = per day as an average over the periods
12= amount of carrots consumed per day in grams
13 = remarks
14= dose of 75 M. E. Radiothorium + 5 mg active iron oxide per kilogram from July 27 to August 21.

1= Table VIIIa (continuation)
2= date 3= body weight in grams 4= amount of urine in ccm
5= urine C in grams 6= urine N in grams 7= C:N
8= urine C in grams 9= urine N in grams 10= C:N
11= per day as an average over the periods
12= amount of carrots consumed per day in grams
13= remarks
14= dose of 75 M. E. radiothorium + 5 mg active iron oxide per kilogram from July 27 to Sept. 7.

1 = Table VIIIa (continuation)

2= date 3= body weight in grams 4= amount of urine in ccm
5= urine C in grams 6= urine N in grams; 7= C:N
8= urine C in grams 9= urine N in grams 10= C:N
11= per day as an average over the periods
12= amount of carrots consumed per day in grams
13= remarks
14= after-period
15= urine poor

1= Table VIIIa (continuation)
As an average over the periods

2 = time 3= body weight in grams 4= amount of food consumed
5= C 6= N 7= C:N 8= remarks
9= iron and radiothorium

1 = Table IXa. Test by Goldbloom on rabbit no. 3 of Table II; carrot feed

2= date 3= body weight in grams 4= amount of urine in ccm
5= urine C in grams 6= urine N in grams 7= C:N
8= urine C in grams 9= urine N in grams 10= C:N
11= amount of carrots consumed per day in grams
12= per day as an average over the periods
13= remarks
14= prep-period
15= Dose of 75 M.E. radiothorium + 5 mg active iron oxide per kilogram and per day from August 26 to Sept. 6

1= Table IXa (continuation)

2= date 3= body weight in grams 4= amount of urine in ccm
5= urine C in grams 6= urine N in grams 7= C:N
8= urine C in grams 9= urine N in grams 10= C:N
11= amount of carrots consumed per day in grams
12= per day as an average over the periods
13= remarks
14= Dose of 75 M. E. radiothorium + 5 mg active iron oxide per kilogram add per day from Sept. 7 to Sept. 19
15= Dose of 75 M.E. radiothorium + 5 mg active iron oxide per kilogram and per day from Sept. 20 to Sept. 29. Some diarrhea.

1 = Table IXa (continuation)

2= date 3= body weight in grams 4= amount of urine in ccm
5= urine C in grams 6= urine N in grams 7= C:N
8= urine C in grams 9= urine N in grams 10= C:N
11= amount of carrots consumed per day in grams
12= remarks
13= per day as an average over the periods
14= after-period

1= Average over the periods

2= time 3= body weight in grams 4= amount of food consumed in grams
5= C 6= N 7= C:N 8= time 9= body weight in grams
10= amount of food consumed in grams 11= C 12= N 13= C:N
14= August 12 to August 25 15= August 26 to Sept. 6
16= Sept. 7 to Sept. 19

1 = table IXb.
Blood count in rabbit no. 3 of table II

2= date 3= body weight in grams 4= hemoglobin in %
5= number of red blood corpuscles in millions
6= coloring index
7. white blood count
8= remarks
9= Dose of 150 M. E. (Mache units) of radiothorium + 5 mg active iron oxide per kilogram and per day.
10= no iron
11= as an average over the periods
12- pre-period
13= main period
14= after-period

1= Table X.
Test by Goldbloom on rabbit no. 4 of Table II; carrot feed

2= date 3= body weight in grams 4= hemoglobin in %
5 = number of red blood corpuscles in millions
6= white blood count 7= remarks
8= Dose of 150 M. E. radiothorium + 10 mg active iron oxide daily.
9= Dose of 300 M.E. radiothorium + 10 mg active iron oxide daily
10= no feeding of iron
11= as an average over the periods
12= pre-period
13= main period
14= main period
15 = after-period

1= Table XI. Test by Goldbloom on rabbit no. 5 of Table II; carrot feed

2= date
3= body weight in grams
4= hemoglobin in %
5= number of red blood corpuscles in millions
6= white blood count
7= coloring index
8= remarks
9= pre-period
10= Dose of 150 M. E. radiothorium + 10 mg active iron oxide daily
11= Dose of 300 M.E. radiothorium + 10 m active iron oxide daily
12= no feeding of iron
13= average over the periods
14= pre-period
15=/main period
16= main period
17= after period

1= Table XII. Test by Goldbloom on rabbit no. 6 of table II; carrot feed

2= date
3= bddy weight in grams
4= hemoglobin in %
5= number of red blood corpuscles in millions
6= white blood count
7= coloring index
8= remarks
9= no feeding of iron
10= Dose of 150 M.E. radiothorium + 10 mg active iron oxide daily
11 = Dose of 300 M. E. radiothorium + 10 mg active iron oxide daily
12= no feeding of iron
13= died
14= average over the periods
15= pre-period
16= main period
17= main period
18= after period

Tabelle I.

Eisendosierung pro Tier und Tag	Beobachtungszeit in Tagen	Zahl der roten Blutkörper im Durchschnitt der Periode
Kaninchen 1.		
0	7	6 484 000
0,005 g (aktives Eisen per os)	8	6 440 000
0,03 g („ „ „ „)	7	5 520 000
0,015 g („ „ subkutan)	8	5 240 000
Kaninchen 2.		
0	8	5 745 000
0,005 g (aktives Eisen per os)	10	5 880 000
0,03 g („ „ „ „)	6	5 760 000
0,015 g („ „ subkutan)	8	5 976 000
Kaninchen 3.		
0	6	5 930 000
0,005 g (inaktives Eisen per os)	8	6 085 000
0,03 g („ „ „ „)	5	5 874 000
0,015 g (aktives „ „ „)	6	5 146 000
Kaninchen 4.		
0	8	6 434 000
0,005 g (inaktives Eisen per os)	10	6 283 000
0,03 g („ „ „ „)	5	5 800 000
0,015 g (aktives „ „ „)	6	4 613 000

Tabelle II.

Eisen- und Thoriumdosierung pro kg Körpergewicht und Tag bei peroraler Zufuhr	Beobachtungszeit in Tagen	Zahl der roten Blutkörper im Perioden-durchschnitt	C:N im Perioden-durchschnitt
Kaninchen 1.			
0	16	5 450 000	1.769
75 Mache-Einheiten, Radiothorium + 5 mg aktives Eisenoxyd	22	5 550 000	2,492
0	—	4 935 000	2.176
Kaninchen 2.			
0	—	5 550 000	1.876
75 Mache-Einheiten, Radiothorium + 5 mg aktives Eisenoxyd	—	5 222 000	2.297
0	—	6 090 000	1.888
Kaninchen 3.			
0	—	4 800 000	1.758
75 Mache-Einheiten, Radiothorium + 5 mg aktives Eisenoxyd	—	4 200 000	1.909
0	—	3 800 000	1,818
Kaninchen 4.			
0	—	5 450 000	—
75 Mache-Einheiten, Radiothorium + 5 mg aktives Eisenoxyd	—	4 490 000	—
150 Mache-Einheiten, Radiothorium + 5 mg aktives Eisenoxyd	—	5 540 000	—
0	—	4 280 000	—
Kaninchen 5.			
0	—	4 600 000	—
75 Mache-Einheiten, Radiothorium + 5 mg aktives Eisenoxyd	—	4 820 000	—
150 Mache-Einheiten, Radiothorium + 5 mg aktives Eisenoxyd	—	5 130 000	—
0	—	5 110 000	—
Kaninchen 6.			
0	—	4 [illegible] 000	—
75 Mache-Einheiten, Radiothorium + 5 mg aktives Eisenoxyd	—	4 680 000	—
150 Mache-Einheiten, Radiothorium + 5 mg aktives Eisenoxyd	—	5 220 000	—
0	—	4 680 000	—

Tabelle III.

Versuch von *Brodski* am Kaninchen Nr. 1: Mohrrübenfutter.

Datum	Gewicht g	Hämoglobin %	Rote Blutkörperchenzahl Millionen	Weiße Blutzählung
	25. V. bis 1. VI.: Ohne Eisen.			
25. V.	1600	80	6,44	6 600
27. V.	1590	74	5,93	7 900
29. V.	1640	70	6,30	11 700
31. V.	1620	74	6,48	10 400
1. VI.	1620	71	7,02	7 700
	2. VI. bis 10. VI.: 0,005 g aktives Eisen täglich.			
2. VI.	1525	75	6,07	8 000
3. VI.	1600	67	5,80	8 400
4. VI.	1600	78	7,40	10 700
5. VI.	1580	75	7,57	11 700
7. VI.	1580	78	6,60	9 400
8. VI.	1610	67	5,31	9 600
9. VI.	1575	71	6,80	12 800
10. VI.	—	—	—	—
	11. VI. bis 16. VI.: 0,03 g aktives Eisenoxyd täglich.			
11. VI.	—	—	—	—
12. VI.	1705	64	6,77	10 700
13. VI.	—	75	5,37	14 000
14. VI.	1630	65	5,71	11 900
15. VI.	1710	70	6,04	13 700
16. VI.	1630	70	5,98	12 900
	17. VI. bis 24. VI.: 0,015 g aktives Eisenoxyd subkutan.			
17. VI.	—	—	—	—
18. VI.	—	—	—	—
19. VI.	—	70	6,07	17 800
20. VI.	—	—	—	—
21. VI.	1480	66	6,08	13 500
22. VI.	1440	60	5,42	12 900
23. IV.	1490	59	4,23	16 400
24. IV.	—	58	4,90	17 200

Versuch von *Brodski* am Kaninchen Nr. 2: Mohrrübenfutter.

Datum	Gewicht g	Hämoglobin %	Rote Blutkörperchenzahl Millionen	Weiße Blutzählung
	25. V. bis 1. VI.: Vorperiode. Ohne Eisen.			
25. V.	1870	72	6,03	8 400
26. V.	1860	78	5,93	9 600
28. V.	1805	70	6,07	6 700
29. V.	1820	72	5,90	9 200
31. V.	1920	72	5,66	9 400
1. VI.	1900	71	5,40	9 100
	2. VI. bis 11. VI.: 0,005 g aktives Eisenoxyd täglich.			
2. VI.	1790	70	5,05	8 300
3. VI.	1830	68	4,98	6 400
4. VI.	1870	67	5,56	6 714
5. VI.	1855	68	6,04	8 300
7. VI.	1820	64	6,37	7 000
8. VI.	1900	64	6,70	7 200
9. VI.	1810	66	6,65	8 100
11. VI.	1920	70	5,88	13 800
	11. VI. bis 16. VI.: 0,03 g aktives Eisenoxyd täglich.			
12. VI.	1960	62	6,22	8 100
13. VI.	—	—	—	—
14. VI.	1970	65	5,59	7 000
15. VI.	1850	61	5,40	9 900
16. VI.	1830	61	5,83	6 800
	17. VI. bis 24. VI.: 0,015 g aktives Eisenoxyd subkutan.			
18. VI.	—	62	5,65	10 200
19. VI.	—	—	—	—
20. VI.	1360	68	6,03	5 300
21. VI.	1270	62	5,90	9 800
22. VI.	1250	58	5,96	8 200
23. VI.	—	61	6,24	9 200
24. VI.	—	—	5,97	—

Tabelle V.

Versuch von *Brodski* am Kaninchen Nr. 3; Mohrrübenfutter.

Datum	Gewicht g	Hämoglobin %	Rote Blutkörperchenzahl Millionen	Weiße Blutzählung	Bemerkungen
25. V.	1700	76	6,18	9 000	Ohne Eisenoxyd
	1690	67	6,77	10 300	
26. V.	1685	70	5,55	9 200	
27. V.	1690	80	5,93	8 900	
28. V.	1660	70	5,57	11 400	
29. V.	1700	72	6,00	10 500	
31. V.	1720	70	5,75	12 100	
2. VI.					
2. VI.	1690	72	5,96	9 000	0,005 g *inaktives* Eisenoxyd täglich per os
3. VI.	1680	69	5,64	10 900	
4. VI.	1670	68	6,44	10 100	
5. VI.	1660	70	6,25	12 300	
7. VI.	1585	70	6,76	11 800	
9. VI.	1590	70	5,59	10 000	
11. VI.	1560	69	5,96	14 600	
12. VI.	1700	69	6,43	11 000	0,03 g *inaktives* Eisenoxyd täglich per os
14. VI.	1630	62	6,11	9 100	
15. VI.	1719	61	5,47	10 700	
16. VI.	1666	60	5,47	10 200	
18. VI.					
21. VI.	1650	60	4,97	11 200	0,015 g *aktives* Eisenoxyd täglich per os
22. VI.	1620	59	4,95	11 800	
23. VI.	1629	59	5,52	8 400	

Tabelle VI.

Versuch von *Brodski* am Kaninchen Nr. 4; Mohrrübenfutter.

25. V.	1690	77	4,89	11 700	Ohne Eisenoxyd
28. V.	1700	73	6,49	15 600	
29. V.	1670	70	6,80	11 300	
31. V.	1670	70	6,20	11 000	
1. VI.	1670	70	6,10	11 600	
2. VI.					
2. VI.	1650	72	6,02	10 000	0,005 g *inaktives* Eisenoxyd täglich per os
3. VI.	1670	70	5,97	9 800	
4. VI.	1630	70	6,31	9 200	
5. VI.	1610	69	5,92	9 300	
7. VI.	1560	71	6,87	9 600	
9. VI.	1590	67	5,57	10 900	
11. VI.	1680	70	6,34	8 900	
11. VI.					
12. VI.	1770	71	5,84	6 400	0,03 g *inaktives* Eisenoxyd täglich per os
14. VI.	1640	58	6,30	9 300	
15. VI.	1680	61	5,86	9 800	
16. VI.	1640	57	5,56	7 500	
17. VI.					
21. VI.	1280	68	4,90	17 000	0,015 g *aktives* Eisenoxyd täglich per os
22. VI.	1210	62	5,06	10 300	
23. VI.	1220	59	4,48	13 300	

Datum	Körpergewicht g	Harnmenge ccm	Harn C g	Harn N g	C : N	Verzehrte Rübenmenge in g pro Tag	Vorperiode	Bemerkungen
23. VII.	2210	390	0,7746	0,2489	3,099	640		
24. VII.	2230	450	0,8523	0,2935	3,020	610		
25. VII.	2250	370	0,7696	0,2608	2,94	610		
26. VII.	2210	410	1,0432	0,2618	4,00	610		
27. VII.	2300	400	0,9000	0,3060	3,02	680		
28. VII.	2330	450	1,0846	0,3316	3,02	750		
29. VII.	2250	460	0,8118	0,3864	2,17	700		
30. VII.	2300	410	0,8048	0,4018	2,01	—		
31. VII.	2350	510	0,9140	0,5472	1,67	880		
1. VIII.	2300	480	0,9636	0,5370	1,79	760		
2. VIII.	2330	410	0,9936	0,5712	1,74	750		
3. VIII.	2350	360	0,8824	0,6116	1,44	610		
4. VIII.	2350	340	0,8556	0,4346	1,96	620		
5. VIII.	2360	340	0,8556	0,4346	1,96	630		
6. VIII.	2250	460	0,8118	0,3864	2,17	700		
7. VIII.	2300	410	0,8048	0,4018	2,01	—		
8. VIII.	2380	510	0,9140	0,5472	1,67	880		
9. VIII.	2350	480	0,9636	0,5370	1,79	760		
10. VIII.	2330	410	0,9936	0,5712	1,74	750		
11. VIII.	2360	360	0,8824	0,6116	1,44	610		
12. VIII.	2350	340	0,8556	0,4346	1,96	620		
13. VIII.	2360	340	0,8556	0,4346	1,96	630		
14. VIII.	2100	390	0,7746	0,2489	3,099	690		
15. VIII.	2100	450	0,8523	0,2935	3,020	750		
16. VIII.	2150	370	0,7696	0,2608	2,94	600		
17. VIII.	2150	410	1,0432	0,2618	4,00	610		
19. VIII.	2150	400	0,9000	0,3060	3,02	660		
20. VIII.	2160	450	1,0846	0,3316	3,02	750		
21. VIII.	2160	460	0,8118	0,3864	2,17	700		
22. VIII.	2210	410	0,8048	0,4018	2,01	—		
23. VIII.	2230	510	0,9140	0,5472	1,67	880		
24. VIII.	2250	480	0,9636	0,5370	1,79	760		
25. VIII.	2210	410	0,9936	0,5712	1,74	750		
26. VIII.	2300	360	0,8824	0,6116	1,44	610		
27. VIII.	2330	340	0,8556	0,4346	1,96	620		
28. VIII.	2350	340	0,8556	0,4346	1,96	630		

Gabe von 7,5 g Radiothorium + 2,5 g aktives Eisenoxyd pro Kaninchen in Tag Nr. 6 VII. – 8. IX. einschl.

Tabelle VII a (Fortsetzung).

Datum	Körpergewicht g	Harnmenge ccm	Harn-C g	Harn-N g	C : N	Harn-C g pro Tag im Periodendurchschnitt	Harn-N g pro Tag im Periodendurchschnitt	C : N im Periodendurchschnitt	Verzehrte Rübenmenge in g pro Tag	Bemerkungen
21. VIII.	2480	890	0,7714	0,2192	2,59				520	
22. VIII.	2490	880	0,7695	0,3003	2,69				700	
23. VIII.	2470	860	0,8280	0,3021	2,76				510	
24. VIII.	2470	880	1,0181	0,3640	2,80				660	
25. VIII.	2480	850	0,9765	0,3920	2,50				580	
26. VIII.	2410	870	0,9369	0,3676	2,69				550	
27. VIII.	2530	460	0,9216	0,3512	2,70				800	
28. VIII.	2480	510	0,8619	0,3219	2,70	0,8865	0,3395	2,6110	760	Gabe von 75 M.-E. Radiothorium i. 5 l aktives Eisenwasser pro Kilogramm KG. Tag. vom 6. VIII. bis 8. IX. einschließlich
29. VIII.	2490	410	0,9758	0,4305	2,260				580	
30. VIII.	2500	360	0,9468	0,4536	2,090				650	
31. VIII.	2460	460	0,9522	0,3864	2,464				680	
1. IX.	2490	420	0,8788	0,3528	2,500				600	
2. IX.	2520	360	0,8280	0,3024	2,705				760	
3. IX.	2470	420	0,7896	0,2646	2,980				650	
4. IX.	2500	280	0,9408	0,3136	3,000				630	
5. IX.	2520	280	0,9576	0,3185	3,005				630	
6. IX.	2460	290	0,9919	0,3451	2,874				500	
7. IX.	2490	330	0,8283	0,3581	2,313				610	
8. IX.	2510	420	0,8403	0,4116	2,156	0,8678	0,3520	2,4329	730	

Tabelle VII a (Fortsetzung).

Datum	Körpergewicht g	Harnmenge ccm	Harn-C g	Harn-N g	C : N	Harn-C g pro Tag im Periodendurchschnitt	Harn-N g pro Tag im Periodendurchschnitt	C : N im Periodendurchschnitt	Verzehrte Rübenmenge in g pro Tag	Bemerkungen
9. IX.	2450	500	0,8700	0,4200	2,0710				740	Nachperiode
10. IX.	2410	350	0,6510	0,2450	2,6570				690	
11. IX.	2490	460	0,8970	0,3220	2,7857				760	
12. IX.	2450	430	0,9159	0,3612	2,5359				700	
13. IX.	2490	380	1,1818	0,4788	2,4661				670	
14. IX.	2540	450	0,9315	0,3780	2,4610				680	
15. IX.	2560	570	0,9130	0,3570	2,5710	0,9236	0,3660	2,5234	750	
16. IX.	2530	450	0,9315	0,6390	1,4786				880	
17. IX.	2510	380	0,8436	0,6334	1,3214				610	
18. IX.	2510	390	0,8424	0,6096	1,4025				680	
19. IX.	2520	560	1,0836	0,6304	1,5050				620	
20. IX.	2510	350	1,0185	0,4410	2,5670				650	
21. IX.	2520	410	1,1135	0,5740	1,9235				700	
22. IX.	2550	390	1,0725	0,5460	1,9615	0,9722	0,6443	1,5082	620	
23. IX.	2510	380	0,6802	0,2660	2,556				570	
24. IX.	2530	280	0,8064	0,2352	3,428				530	
25. IX.	2540	290	0,8613	0,2871	3,000				580	
26. IX.	2540	360	0,8496	0,3162	2,760				700	
27. IX.	2520	330	0,7854	0,2772	2,800				630	
28. IX.	2560	320	0,8096	0,2688	3,000				560	
29. IX.	2580	250	0,7025	0,3528	2,000				600	
30. IX.	2490	250	0,7250	0,4200	1,725	0,7815	0,3130	2,4967	500	

Tabelle VII a. (Fortsetzung.)

Im Periodendurchschnitt.

Zeit	Körpergewicht g	Verzehrte Nahrungsmenge g	C	N	C : N	Bemerkungen
27. VII. bis 5. VIII.	2277	681	0,8674	0,4923	1,7601	
6. VIII. „ 13. VIII.	2336	710	0,8654	0,4791	1,8065	
14. VIII. „ 20. VIII.	2411	668	0,9090	0,2914	3,1192	Eisen und Radiothorium
21. VIII. „ 28. VIII.	2457	627	0,8865	0,3805	2,6110	
29. VIII. „ 8. IX.	2491	689	0,8078	0,3329	2,4329	
9. IX. „ 15. IX.	2450	698	0,9286	0,3660	2,5284	
16. IX. „ 22. IX.	2521	680	0,9722	0,6443	1,5089	
23. IX. „ 30. IX.	2518	583	0,7875	0,3130	2,4967	

Tabelle VII b.

Blutzählung bei dem Kaninchen Nr. 1 der Tabelle II.

Datum	Körpergewicht g	Hämoglobin %	Rote Blutkörperchenzahl Millionen	Farbeindex	Weiße Blutzählung	Bemerkungen
2. VII.	2210	70	5,03	0,02	5800	Vorperiode — ohne Verfütterung von Eisen
8. VII.	2210	75	5.09	0,75	5200	
17. VII.	2200	75	5.57	0,68	5400	
5. VIII.	2220	80	5.67	0,71	8200	
10. VIII.	2330	80	6.03	0.66	8600	Gabe v. 75 Mgm. Radiothorium + 5 mg reines Eisenoxyd pro Kilogramm u. Tag
17. VIII.	2430	80	5.45	0.74	6000	
6. IX.	2460	85	5.12	0,82	7000	
16. IX.	2530	80	5.15	0,78	7600	Nachperiode
24. IX.	2530	80	5.24	0.77	5400	
1. X.	2500	80	4,42	0,80	6800	

Im Periodendurchschnitt.

Vorperiode	2277	75	5,49	0,69	6150
Hauptperiode	2424	82	5,55	0,78	7460
Nachperiode	2496	80	4.93	0,82	6400

Datum	Körpergewicht g	Harmenge ccm	HarnC g	HarnN g	C : N	Verzehrte Kohlenhydratenergie in g pro Tag	Bemerkungen
			pro Tag im Periodendurchschnitt				
27. VI.	2480	320	0,551	0,537	1,585	687	Vorperiode
28. VI.	2400	430	0,554	0,542	1,676	626	
29. VI.	2370	400	0,656	0,448	1,461	666	
30. VI.	2460	530	0,813	0,403	2,695	820	
1. VII.	2420	420	0,709	0,470	1,572	650	
2. VII.	2450	480	0,661	0,561	1,177	650	
3. VII.	2400	360	0,674	0,579	1,341	710	
4. VII.	2450	480	1,031	0,611	1,677	750	
5. VII.	2430	410	0,746	0,659	1,134	700	
6. VII.	2450	440	0,749	0,646	1,159	700	
7. VII.	2450	440	0,755	0,651	1,159	666	
8. VII.	2420	380	1,403	0,955	1,521	625	
9. VII.	2410	490	0,761	0,720	1,062	881	
10. VII.	2450	390	0,875	0,628	1,971	676	
11. VII.	2450	410	0,791	0,688	1,150	530	Gabe v. 75 Mgm. Radiothorium + 5 mg reines Eisenoxyd pro Kilogramm u. Tag
12. VII.	2400					540	
13. VII.	2380						
14. VII.							
15. VII.							
16. VII.							
17. VII.							
18. VII.	2320	250	0,673	0,375	1,79	520	
19. VII.	2370	405	1,072	0,599	1,79	600	
20. VII.	2350	330	0,967	0,491	1,97	550	
21. VII.	2330					500	
22. VII.	2330					480	
23. VII.	2280	340	0,8806	0,3895	2,21	550	
24. VII.	2300	310	0,862	0,363	2,33	520	
25. VII.	2300	380	1,071	0,479	2,23		

Tabelle VIII a (Fortsetzung).

Datum	Körpergewicht g		Harnmenge ccm	Harn-C g	Harn-N g	C : N	Harn-C g	Harn-N g	C : N	Verzehrte Nahrungsmenge in g pro Tag		Bemerkungen
							pro Tag im Periodendurchschnitt					
26. VII.												
27. VII.	2300		290	0,716	0,325	2,20				550		
28. VII.	2280		320	0,960	0,425	2,14				520		
29. VII.	2220	2253	290	0,832	0,365	2,28	2,0325			660		
30. VII.	2240		300	0,717	0,409	1,75				450	537	
31. VII.	2200		360	0,903	0,403	1,99				550		
1. VIII.	2250		330	0,812	0,365	2,42				600		
2. VIII.	2260		350	0,983	0,291	3,31				570		
							0,8245	0,3651	2,2581			
3. VIII.	2240		350	0,843	0,355	2,39				540		
4. VIII.	2240		340	0,749	0,390	1,92				600		
5. VIII.	2220		310	1,020	0,369	2,76				620		
8. VIII.	2270	2278	510	1,2546	0,5712	2,20	2,092			660		Gabe von 75 ccm Rainotherm i. St.
9. VIII.	2330		260	0,5876	0,4004	1,46				560	524	aktives Eiweiß
10. VIII.	2270		520	1,2376	0,6552	1,90				650		pro Kilogramm vom
11. VIII.	2310		360	1,0221	0,5511	1,90				610		27. VII. bis 21. VIII.
12. VIII.	2270		350	0,9811	0,5520	1,80				640		
13. VIII.	2310		230	0,7252	0,3538	2,05				480		
							0,9856	0,4674	2,0917			
14. VIII.	2310		360	1,1239	0,3276	3,430				530		
15. VIII.	2330		360	1,0258	0,3728	2,750				590		
16. VIII.	2350		340	1,0231	0,3538	2,707				570		
18. VIII.	2320	2332	330	1,0095	0,3231	3,123	2,4358			540	312	
19. VIII.	2320		296	0,9680	0,3151	2,960				500		
20. VIII.	2370		350	1,0010	0,3439	3,000				550		
21. VIII.	2360		350	1,3310	0,4119	3,060				410		
							1,0579	0,3591	2,9459			

Tabelle VIII a (Fortsetzung).

Datum	Körpergewicht g		Harnmenge ccm	Harn-C g	Harn-N g	C : N	Harn-C g	Harn-N g	C : N	Verzehrte Nahrungsmenge in g pro Tag		Bemerkungen
							pro Tag im Periodendurchschnitt					
22. VIII.	2400		370	1,1692	0,4692	2,603				410		
23. VIII.	2300		320	1,0912	0,5076	2,368				350		
24. VIII.	2300		310	1,0737	0,5612	2,060				590		
25. VIII.	2300	2326	330	1,0329	0,4158	2,420	2,0743			480	512	
26. VIII.	2330		330	1,0681	0,4920	2,301				560		
27. VIII.	2360		420	0,9038	0,4410	2,185				556		
28. VIII.	2450		430	0,9750	0,3512	2,760				620		
							1,0031	0,44683	2,2195			
29. VIII.	2380		400	1,0840	0,5140	2,1400				640		
30. VIII.	2290		350	1,1655	0,5040	2,4000				480		Gabe von 75 ccm Rainotherm i. St.
31. VIII.	2320		430	1,5975	0,6660	2,5389				600		aktives Eiweiß
1. IX.	2350		340	1,0679	0,3721	2,8700				560		pro Kilogramm vom
2. IX.	2350	2345	360	1,0471	0,4464	2,4091	2,4744			650	524	27. VIII. bis 21. IX.
3. IX.	2350		330	1,0329	0,3231	0,1940				580		
4. IX.	2310		290	0,9541	0,3248	2,9700				540		
5. IX.	2360		370	0,8325	0,3625	2,3050				680		
6. IX.	2360		250	0,8175	0,3150	2,5600				400		
7. IX.	2310		270	0,8129	0,3021	2,6877				500		
							1,0177	0,4036	2,5215			

Tabelle VIIIa (Fortsetzung).

Datum	Körpergewicht g	Harnmenge ccm	Harn-C g	Harn-N g	C:N	Harn-C g	Harn-N g	C:N	Verzehrte Kotmenge pro Tag g	Bemerkungen
			pro Tag im Periodendurchschnitt							
			2238			1,7237				Nachperiode (Harn schlecht
8. IX.	2300	620	1,0660	0,5696	2,0020				700	
9. IX.									400	
10. IX.	2300	260	0,6800	0,3640	1,9000				650	
11. IX.	2300	350	1,2530	0,6350	1,9410				600	
12. IX.	2300	320	1,1392	0,6621	1,6950				420	
13. IX.	2280	290	0,9530	0,5684	1,7110				470	
14. IX.	2310	350	1,1164	0,7092	1,5100				590	
15. IX.	2290	430	1,0363	0,6020	1,7210				550	
16. IX.	2310	400	1,0800	0,6720	1,6071				570	
17. IX.	2280	420	1,0500	0,7056	1,4880				650	
18. IX.	2280	410	0,8856	0,5166	1,7140				580	
19. IX.	2330	300	0,9000	0,4620	1,9485				450	
20. IX.	2300	310	1,1439	0,6076	1,8826	1,0287	0,6211	1,6572		
			2260			2,0510				
21. IX.	2280	360	1,4004	0,7308	2,0350				690	
22. IX.	2310	210	0,8988	0,4162	2,1577				470	
23. IX.	2310	360	0,9900	0,4680	2,1062				600	
24. IX.	2330	310	1,0574	0,4188	2,5240				570	
25. IX.	2270	270	0,8235	0,3360	2,4510				690	
26. IX.	2220	310	0,8804	0,4125	2,1340				520	
27. IX.	2260	280	0,7028	0,3136	2,2410				430	
28. IX.	2230	320	0,9523	0,4032	2,3620				640	
29. IX.	2270	250	0,5656	0,3525	1,7066				600	
30. IX.	2250	400	0,7180	0,3360	2,1369	0,8822	0,4158	2,1218		

Tabelle VIIIa (Fortsetzung).

Im Periodendurchschnitt.

Zeit	Körpergewicht g	Verzehrte Kotmenge g	C	N	C:N	Bemerkungen
27. VI. bis 4. VII.	2417	684	0,7564	0,3247	1,4726	
4. VII. „ 10. VII.	2455	693	0,8127	0,3257	1,2493	
11. VII. „ 25. VII.	2336	582	0,8888	0,4835	1,8396	
26. VII. „ 2. VIII.	2258	557	0,8245	0,3651	2,2781	
3. VIII. „ 13. VIII.	2274	584	0,9270	0,4674	2,0017	Eisen und Radiothorium
14. VIII. „ 21. VIII.	2322	512	1,0379	0,4754	2,0459	
22. VIII. „ 28. VIII.	2250	524	1,0394	0,4283	2,2195	
29. VIII. „ 7. IX.	2242	564	1,0177	0,4736	2,3215	
8. IX. „ 20. IX.	2298	543	1,1287	0,6211	1,8502	
21. IX. „ 30. IX.	2266	509	0,8822	0,4158	2,1218	

Tabelle VIIIb.

Blutzählung bei dem Kaninchen Nr. 2 der Tabelle IV.

Datum	Körpergewicht g	Hämoglobin %	Rote Blutkörperchen Millionen	Färbeindex	Weiße Blutkörperchen	Bemerkungen
2. VII.	2450	80	5,33	0,72	4000	Keine Vergiftung v. Eisen
8. VII.	2420	75	5,41	0,69	5800	
18. VII.	2320	80	5,41	0,74	7200	Gibt 0,75 g Radiothorium + 5 mg aktives Eisen oxydpräparat gramm u. Tag
27. VII.	2300	75	4,51	0,88	7200	
5. VIII.	2220	70	5,33	0,64	6400	
17. VIII.	2320	75	5,43	0,74	5100	
6. IX.	2300	80	5,21	0,71	4700	
16. IX.	2310	85	5,55	0,77	5300	Nachperiode — keine Verfütterung von Eisen
24. IX.	2330	85	6,12	0,69	5900	
1. X.	2280	80	6,16	0,71	6000	

Im Periodendurchschnitt.

Vorperiode	2436	78	5,48	0,70	4900
Hauptperiode	2032	76	5,22	0,73	5300
Nachperiode	2282	33	5,94	0,72	5800

Tabelle IXa. Versuch von *Goldbaum* am Kaninchen Nr. 3 der Tabelle II; Mohrrübenfutter.

Datum	Körpergewicht g		Harnmenge ccm	Harn C g	Harn N g	C : N		Harn C g	Harn N g	C : N	Verzehrte Rübenmenge in g pro Tag		Bemerkungen
								pro Tag im Periodendurchschnitt					
													Vorperiode
12. VIII.	3010		350	1,6030	1,0670	1,550					810		
13. VIII.	2850		500	1,1950	0,4200	2,800					570		
14. VIII.	2870		440	0,9812	0,5514	1,770					660		
15. VIII.	2770		410	1,0742	0,5166	2,078					550		
16. VIII.	2800		420	1,2390	0,5292	2,340					660		
17. VIII.	2780	2749	370	1,2469	0,7770	1,700	1,5748				530	380	
19. VIII.	2700		300	1,1820	0,5460	2,160					460		
20. VIII.	2700		310	1,0664	0,6444	1,700					550		
21. VIII.	2660		280	1,8244	0,6624	1,600					320		
22. VIII.	2660		350	1,2390	0,7840	1,600					720		
23. VIII.	2660		370	1,2099	0,8288	1,500					450		
25. VIII.	2580		310	1,1934	0,7140	1,700		1,2153	0,69115	1,7583	530		
26. VIII.	2560		400	1,4360	0,8100	1,710					670		
27. VIII.	2590		340	1,2716	0,8072	1,570					450		
28. VIII.	2540		350	1,0980	0,5850	1,7100					570		15
29. VIII.	2560		420	1,2348	0,7056	1,750					770		
30. VIII.	2540		470	1,3207	0,7836	1,673					410		Gabe von 15 M.-E. Radiothorium + Sto-
31. VIII.	2560	2570	390	1,2597	0,5460	2,300	2,2295				550	353	aktives Uran pro Kilogramm und
1. IX.	2560		350	1,0080	0,5880	1,800					480		Tag vom 2. VIII.
2. IX.	2660		Kein Harn								660		bis 6. IX.
3. IX.	2630		360	1,0368	0,4264	2,4202					590		
4. IX.	2570		307	1,9840	0,3570	2,7590					480		
5. IX.	2670		380	1,2464	0,5320	2,5440					660		
6. IX.	2510		350	1,0038	0,4178	2,4295		1,2307	0,6599	1,9107	840		

Tabelle IXa (Fortsetzung).

Datum	Körpergewicht g		Harnmenge ccm	Harn C g	Harn N g	C : N		Harn C g	Harn N g	C : N	Verzehrte Rübenmenge in g pro Tag		Bemerkungen
								pro Tag im Periodendurchschnitt					
7. IX.	2460		230	0,8553	0,4186	2,049					350		
8. IX.	2400		Schlecht								220		
9. IX.	2420		290	Mit Blut gemischt							550		
10. IX.	2480		360	1,1626	0,7410	1,570					420		
11. IX.	2460		Schlecht								310		Gabe von 75 M.-E.
12. IX.	2560		410										Radiothorium + Sto-
13. IX.	2570	2490	330	1,0529	0,6237	1,687	2,2508				410	447	aktives Uran pro
14. IX.	2520		440	0,5516	0,3383	3,103					500		Kilogramm und
15. IX.	2460		500	0,8807	0,3511	2,511					580		Tag vom 7. IX. bis
16. IX.	2500		560	1,2050	0,3560	2,870					670		19. IX.
17. IX.	2510		340	1,2760	0,5712	2,252					550		
18. IX.	2500		400	1,1160	0,5663	2,000					570		
19. IX.	250		260	1,0248	0,4466	2,285		1,0685	0,4144	2,1639	470		
20. IX.	2420		180	1,0602	0,5544	1,8770					330		
21. IX.	2350		200	0,9252	0,5056	1,821					300		
22. IX.	2460		260	1,2193	0,6362	1,9160					450		
23. IX.	2380		160	0,8176	0,4180	1,955					320		Gabe von 75 M.-E.
24. IX.	2350	2422	150	0,5015	0,5880	1,5173	2,1753				320	504	Radiothorium + Sto-
25. IX.	2380		190	0,6493	0,4092	1,6 6					400		aktives Uran pro
26. IX.	2360		170	0,6791	0,5256	1,295					470		Kilogramm und
27. IX.	2360		150	0,8870	0,6301	1,190					350		Tag vom 20. IX. bis
28. IX.	2210		Kein Harn								340		29. IX. etwas Durch-
29. IX.	2190		130	0,7423	0,5460	1,360		0,8722	0,5439	1,6039	390		fall.

Tabelle IX a (Fortsetzung).

Datum	Körpergewicht g	Harnmenge ccm	Harn C g	Harn N g	C : N	Harn C g pro Tag im Periodendurchschnitt	Harn N g	C : N	Verzehrte Kostmenge in g pro Tag	Bemerkungen
						Nachperiode				
30. IX.	2150	200	0,9930	0,8960	1,1135				890	
1. X.	2170	240	0,8781	0,7056	1,2445				850	
2. X.	2160	230	0,5681	0,4440	1,2793				870	
3. X.	2100	200	0,4040	0,4040	1,0000				280	
4. X.	2150	210	0,6468	0,6468	1,0000				490	
5. X.	2160	210	0,9115	0,6115	1,4903				340	
6. X.	2130	210	0,8862	0,5972	1,4716				360	
7. X.	2120	260	0,7231	0,5824	1,2517				480	
8. X.	2120	240	0,6768	0,5376	1,2589				840	
9. X.	2105	180	0,5796	0,3780	1,5333				450	
10. X.	2070	250	0,6150	0,4960	1,2551				630	
11. X.	2050	220	0,6754	0,4928	1,3705				400	
12. X.	2010	240	0,9360	0,6656	1,4140				320	
13. X.	2010	200	0,6680	0,5010	1,3189				410	
14. X.	2040	310	0,9883	0,5298	1,744				580	
15. X.	2083	Durchschn.							540	
16. X.	2080	370	0,6883	0,5180	1,135	0,7466	0,5606	1,3310	600	

Im Periodendurchschnitt.

Zeit	Körpergewicht g	Verzehrte Nahrungsmenge g	Harn C g	Harn N g	C : N	Zeit	Körpergewicht g	Verzehrte Nahrungsmenge g	Harn C g	Harn N g	C : N
12. VIII. bis 25. VIII.	2049	589	1,2453	0,6911	1,7583	20. IX. bis 29. IX.	2082	369	0,8722	0,5130	1,6
26. VIII. - 6. IX.	2060	559	1,2000	0,6500	1,8167	30. IX. „ 16. X.	2044	426	0,7466	0,5606	1,33
7. IX. - 15. IX.	2077	614	1,1	0,6	1,8						

Blutzählung am Kaninchen Nr. 3 der Tabelle II.

Datum	Körpergewicht g	Hämoglobin %	Rote Blutkörperchenzahl Millionen	Färbeindex	Weiße Blutkörperchen	Bemerkungen
16. VIII.	3010	70	4,36	0,81	17[illegible]	Gabe von 150 M.-E. Radiothorium + 5 mg aktives Eisenoxydpräparat pro Kilogramm u. Tag.
26. VIII.	2550	75	4,05	0,83	6600	
6. IX.	2510	80	4,07	0,86	4200	
17. IX.	2510	70	—	—	5400	
24. IX.	2380	85	3,25	1,06	42[illegible]	
4. X.	2150	81	3,80	1,09	8800	Kein Eisen.
Im Periodendurchschnitt.						
Vorperiode	2749	70	4,56	0,81	4800	
Hauptperiode	2440	77	4,03	0,89	5200	
Nachperiode	2084	80	3,86	1,06	8850	

Tabelle X.

Versuch von *Goldblum* am Kaninchen Nr. 4 der Tabelle II; Mehrfütterung.

Datum	Körpergewicht g	Hämoglobin %	Rote Blutkörperchenzahl Millionen	Weiße Blutkörperchen	Bemerkungen
12. VII.	1750	69—70	5,15	8[illegible]	
13. VII.					Gabe von 80 M.-E. Radiothorium
19. VII.		70	—	4[illegible]	+ 5 mg aktives
27. VII.		70	4,29	[illegible]	Eisenoxyd täglich.
4. VIII.		73	4,70	5[illegible]	
10. VIII.	1740	73	4,51	8[illegible]	
10. VIII.					Gabe von 80 M.-E. Radiothorium
12. VIII.		86	5,04	[illegible]	+ 5 mg aktives
16. VIII.	1740	86	5,29	[illegible]	Eisenoxyd täglich.
20. VIII.	1870	80	5,62	[illegible]	
26. VIII.	1850	80	5,01	[illegible]	
31. VIII.	1880	80	—	[illegible]	
6. IX.	1870	85	5,27	8[illegible]	
7. IX.					Keine Verfütterung von Eisen
15. IX.	1899	85	4,62	4[illegible]	
24. IX.		85	4,24	5[illegible]	
Im Periodendurchschnitt.					
Vorperiode	1750	65	5,46	[illegible]	
Hauptperiode	1749	78	4,49	[illegible]	
Hauptperiode	1828	81	5,54	5[illegible]	
Nachperiode	1890	85	4,28	5[illegible]	

Tabelle XI. Versuch von *Goldbloom* am Kaninchen Nr. 5 der Tabelle II; Mohrrübenfutter.

Datum	Körpergewicht g	Hämoglobin %	Rote Blutkörperchenzahl Millionen	Weiße Blutzählung	Färbeindex	Bemerkungen
12. VII.	1810	70	4,00	4700	0,87	Vorperiode
19. VII.		70	3,17	3400	0,88	Gabe v. 150 M.-E.
20. VII.		75	4,03	6100	0,76	Radiothorium
4. VIII.		75	4,00	5870	0,81	+ 10 mg aktives
10. VIII.	1900	75	4,61	6100	0,81	Eisenoxyd täglich.
10. VIII.						Gabe v. 200 M.-E.
12. VIII.		80	4,14	6000	0,87	Radiothorium
16. VIII.	1900	75	6,06	5800	0,62	+ 10 mg aktives
20. VIII.	2000	85	3,79	4,50	0,75	Eisenoxyd täglich.
25. VIII.	2100	75	5,62	5800	0,67	
31. VIII.	2150	80	5,28	5570	0,74	
6. IX.	2170	80	3,79	5000	1,00	
7. IX.	2200					Keine Verabreichung v. Eisen
15. IX.	2300	85	4,57	5400	0,91	
24. IX.	—	80	5,65	5200	0,71	
			Im Periodendurchschnitt.			
Vorperiode .	1810	70	4,09	4700	0,87	
Hauptperiode	1900	74	4,85	5950	0,76	
Hauptperiode	2080	79	5,13	5573	0,79	
Nachperiode.	2250	83	5,11	5600	0,85	

Tabelle XII. Versuch von *Goldbloom* am Kaninchen Nr. 6 der Tabelle II; Mohrrübenfutter.

Datum	Körpergewicht g	Hämoglobin %	Rote Blutkörperchenzahl Millionen	Weiße Blutzählung	Färbeindex	Bemerkungen
8. VII.	1780	75	4,24	5400	0,89	Keine Verabreichung v. Eisen
12. VII.		75	4,18	5400	0,91	
13. VII.						Gabe v. 150 M.-E.
19. VII.		75	3,85	5500	0,78	Radiothorium
27. VII.		75	4,54	5100	0,83	+ 10 mg aktives
4. VIII.		75	4,23	4200	0,89	Eisenoxyd täglich.
10. VIII.	1380	75	3,71	3000	1,00	
10. VIII.	1680					Gabe v. 200 M.-E.
16. VIII.	1780	80	6,28	8900	0,63	Radiothorium
20. VIII.	1800	80	—	8400	—	+ 10 mg aktives
26. VIII.	1930	80	5,71	8800	0,71	Eisenoxyd täglich.
31. VIII.	2000	80	4,35	5100	0,92	
6. IX.	1900	80	4,51	4700	0,88	
14. IX.	1700	75	4,08	4900	0,92	Keine Verabreichung v. Eisen
22. IX.			Gestorben			
			Im Periodendurchschnitt.			
Vorperiode .	1780	75	4,21	5400	0,90	
Hauptperiode	1880	75	4,08	5125	0,86	
Hauptperiode	1850	80	5,22	4150	0,79	
Nachperiode.	1700	75	4,08	4900	0,88	

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EXPERIMENTAL AND CLINICAL COMPARATIVE STUDY OF IRON CARBOHYDRATE COMPLEX† WITH FERROUS GLUCONATE

K.C. Gupta,° S.V. Mulgund,° P.V. Karandikar,°°° V.P. Valame, A.B. Vaidya,°°° M.J. Shah°° and U.K. Sheth.°

The response of a patient suffering from iron deficiency anemia to iron therapy is one of the most satisfactory experiences of a practising physician. The most convenient way of administration of iron is the oral route. There are many different iron preparations available in the market. The basis of all such preparations is a ferrous salt, there being little difference if any at all, amongst them. The commonest ferrous salts in use are:—ferrous sulphate, ferrous fumarate, ferrous gluconate, ferrous succinate *etc.* There are, however, some differences in the side-effects produced. Irritation of the gastrointestinal tract is experienced by most patients to a greater or lesser degree, the incidence varying with the salts used. Ferric salts which produce less of local irritation have fallen into disuse, mainly because their absorption is quantitatively far less than an equivalent amount of a ferrous salt.[1]

In an effort to reduce the local irritative action of the ferrous salts on the gastrointestinal tract, many compounds have been market where the iron is "bound" or "chelated" with an organic complex. Such iron complexes are known as chelated iron compounds. This paper presents the results obtained with one such compound, an iron carbohydrate complex with glycine containing 50 mg. of elemental iron per 5 ml.† This preparation was administered in a syrup form. As a standard of comparison, ferrous gluconate in syrup form containing an equivalent amount of elemental iron was used.

The iron in this iron carbohydrate complex is present mainly in the ferric form.

METHODS

(i) *Experimental studies:*

Absorption studies in healthy rabbits.

Serum iron levels were estimated in 2 groups of rabbits, before and after administration of the iron compounds. Each group consisted of 7 rabbits.

Gr. I received the iron carbohydrate complex.

Gr. II received the ferrous gluconate syrup.

Serum iron estimations were done by the dipyridyl method of Ramsay.[3] In this method, proteins are removed by heating in boiling water and then centrifuging or filtering. A solution of dipyridyl in acetic acid is added to serum followed by a reducing agent. Ferrous iron gives a pink colour with 2.2'-dipyridyl.

From the Departments of Pharmacology° and Medicine,°°, Seth. G.S Medical College and K.E.M. Hospital, Parel, Bombay-12 and Clinical Drug Trial Unit C.S.I.R.°°°

†. Iron Carbohydrate Complex was supplied by Unichem Ltd. Bombay-26

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(ii) Studies on rabbits rendered experimentally anaemic by bleeding:

Twelve albino rabbits weighing 1.2 Kg. to 1.5 Kg. were rendered anemic by removal of half their estimated blood volume.

Serum iron and hemoglobin were determined. These 12 rabbits were then divided into 2 groups of 6 each.

Gr. I—received iron carbohydrate complex, the dosage being 30 mg. elemental iron/Kg. body wt. per day for 3 weeks.

Gr. II—received ferrous gluconate syrup, the dosage being identical.

At the end of the trial period, serum iron and hemoglobin were again determined.

(iii) *Determination of LD 50 in mice:—*

LD 50 studies in mice were done using ferrous gluconate and iron carbohydrate complex according to the method used by Eickholt and White.[2]

Mice were fasted for at least 20 hours and never more than 24 hours, with water *ad lib.* The medication was given entirely by the oral route via stomach intubation. The doses of iron compounds were calculated on a milligram per kilogram weight basis. The number of animals dead after 24 hours was observed.

(iv) *Absorption studies in healthy normal human volunteers:—*

Eight normal healthy adults were selected. Each received iron carbohydrate complex containing 100 mg. of elemental iron orally. Serum iron was estimated in the fasting state and 3 hours after administration of the iron carbohydrate complex. Fifteen days later, the same individuals received ferrous gluconate syrup containing an equivalent amount of elemental iron and similar serum iron estimations were done so as to enable a comparison between the absorption of the iron carbohydrate complex and the ferrous gluconate syrup.

(v) *Studies in patients of iron deficiency anaemia:—*

Forty male patients with iron deficiency anemia were chosen for the trial. Their hemoglobin values were between 3 and 7.5 G. These patients were divided into 2 groups of 20 patients each and a double blind study conducted, whereby 20 patients received iron carbohydrate complex (dose -100 mg. of elemental iron per day orally), the other 20 receiving an identical quantity of ferrous gluconate syrup. Weekly haemograms were carried out and a comparison made between the 2 groups to assess their relative therapeutic efficacy.

RESULTS

Iron absorption studies in normal healthy rabbits (Table 1) and in healthy human volunteers (Table 3) as seen by rise in serum iron before and after the drugs showed that both ferrous gluconate and the test compound produced similar rise in serum iron (Table 1).

In rabbits rendered anemic by bleeding both iron carbohydrate complex and ferrous gluconate showed nearly similar rise in serum iron levels and hemoglobin per cent at the end of 3 weeks treatment (Table 2).

The LD 50 of ferrous gluconate was 101 ± 52 mg./Kg. while that of iron carbohydrate complex could not be determined as the dose proved to be too large to be fed orally.

Results with ferrous gluconate and the test compound in clinical cases of iron deficiency anemia treated for a period of 4 weeks showed nearly similar rise in hemoglobin levels and packed cell volumes. (Table 4 and 5 and Fig. 1).

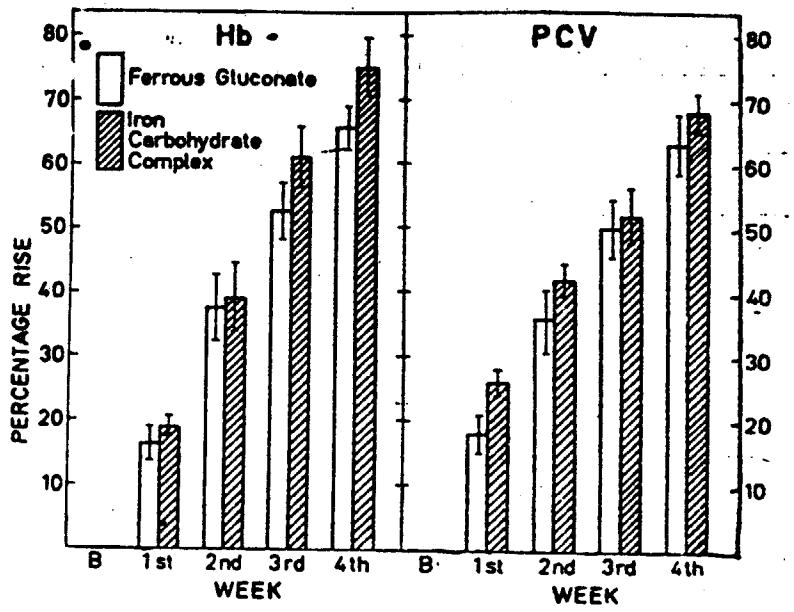

Fig. 1.—Therapeutic response to ferrous gluconate and iron carbohydrate complex in iron deficiency anemia.

The detailed data of individual patients are shown in Table 4 for syrup A and Table 5 for syrup B. The average rise in values of hemoglobin and P.C.V. in two groups did not show any statistically significant difference.

TABLE 1.—*Iron Absorption Studies in Normal Healthy Rabbits.*

(7 rabbits per group-Cross over test)

Drug	Serum iron μ g. per 100 ml.		
	Fasting	After 5 hours	After 24 hours
Ferrous Gluconate	209 S.D.±44 S.E.±17	283 S.D.±43 S.E.±13	217 S.D.±34 S.E.±10
Iron Carbohydrate Complex	194 S.D.±35 S.E.±13	283 S.D.±35 S.E.±13	217 S.D.±42 S.E.±10

There is no statistically significant difference between the two groups.

TABLE 2.—*Serum Iron Levels and Hemoglobin Levels before and after Treatment. (Mean Results) in Rabbits Rendered Anaemic (3 Weeks Treatment)*

	Serum iron μ g.%		Hemoglobin G.%	
	Before therapy	After therapy	Before therapy	After therapy
GROUP I. Iron carbohydrate complex	40	141	5.9	11.5
GROUP II. Ferrous gluconate syrup ...	64	122	5.8	11.8

TABLE 3.—*Absorption Studies in Healthy Human Volunteers.*

Drug	MEAN VALUES Serum Iron		
	Fasting (μg.100 mg.)	3 hours after (μg.100 ml.)	Percentage rise
Iron carbohydrate complex ...	100.62±13	196.5±54	97.5
Ferrous gluconate	109.5±18	209±57	98.5

SIDE EFFECTS

One patient who was being treated with iron carbohydrate complex developed diarrhea during second week of treatment.

Out of 20 patients who were treated with ferrous gluconate, 2 patients developed severe pain in abdomen and diarrhea during the first week, one had vomiting and diarrhea, one complained of only pain in abdomen, while another patient complained of constipation.

DISCUSSION

The efficacy of oral iron in the treatment of iron deficiency anemia is unquestioned. One of the disadvantages of the administration of iron by mouth is the gastrointestinal irritation caused by the iron salts. And oral treatment needs to be continued for several months after the hemoglobin level reaches normal in order to replenish the depleted iron stores. Until recently, inorganic ferrous salts have been used because of their ready absorbability and cheapness. There is very little to choose between the different ferrous salts, as there is hardly any difference amongst them in efficacy, though there is some difference in the side effects which they produce.[1] Organic compounds containing iron in a bound form do not irritate the gastrointestinal mucosa as much as the inorganic salts. This is because the release of iron after ingestion is gradual so that at any one time the concentration of free iron in the gastrointestinal tract is never very high. Some doubts were cast as to their

therapeutic efficacy as it was postulated that Fe *** iron carbohydrate complexes were absorbed poorly from the gastrointestinal tract. However, results both from animal experiments, human volunteers and a comparative double blind trial do not confirm this hypothesis. Iron carbohydrate complex, compares favourably with ferrous gluconate, both as regards absorption and the therapeutic efficacy when given in identical doses. Comparison of the side effects between the 2 groups clearly outlines the fact that iron carbohydrate complex produces far less gastro-intestinal irritation than ferrous gluconate.

TABLE 4.—*Anaemia Trial with Syrup A (Iron Carbohydrate Complex)*

Name	Sex	BEFORE TREATMENT		AFTER TREATMENT							
				1st week		2nd week		3rd week		4th week	
		Hb G.%	PCV%	Hb G.%	PCV%	Hb G%	PCV%	Hb G.%	PCV%	Hb G.%	PCV%
1. K.Y.	M	4	15	6.5	22	7.5	29	11	38	13	42
2. P.V.	M	7.5	28	8	30	8	30	9.5	36	9.5	36
3. R.V.	M	7.5	32	8	33	9	38	10	38	...	...
4. B.H.	M	6	26	6	27	7	32	8	34	8.5	36
5. H.P.	M	5	22	6	24	6.5	25	8	30	10	35
6. B.T.	M	4.5	21	5	25	7	29	9	37	10.5	39
7. R.Z.	M	5	21	5.5	23	6.5	25	8	31	9.5	34
8. T.S.	M	6	23	6.5	25	7.5	30	8	32	9.5	37
9. K.D.	F	4	18	5	20	7	30	9	34	10	37
10. B.L.	M	3	15	4.5	22	5.5	22	7.5	30	9	36
11. L.J.	M	5.5	20	6.5	25	8.5	32	10.5	39	10.5	40
12. M.J.	M	6	25	7	27	8.5	29	8.5	30	8	28
13. H.N.	M	4.5	17	5.5	20	6.5	25	7.5	30	9	30
14. M.S.	M	6.5	20	7	23	10	35	...	...	...	...
15. B.B.	M	5	25	5.5	26	6	28	7.5	35	9.5	39
16. S.M.	F	7	30	8.5	35	9.5	38	...	...	...	...
17. N.M.	M	6	28	6.5	30	...	...	10	43	...	...
18. D.A.	M	4	22	5	25	...	...	5.5	25	6	28
19. S.T.	M	4	20	...	...	5	24	5.5	28	6	30
20. D.J.	M	4.5	21	5.5	25	7.5	30	10	41	...	...

Mean percentage increase	1st week	2nd week	3rd week	4th week
Hb	19 S.E.±3.46	39.13 S.E.±5.83	61.30 S.E.±8.18	75.15 S.E.±12.57
PCV	18 S.E.±6.16	36 S.E.±10.44	50 S.E.±9.4	63 S.E.±9.79

TABLE 5.—*Anaemia Trial with Syrup B (Ferrous Gluconate)*

Name		Sex	Before Treatment		After Treatment							
					1st week		2nd week		3rd week		4th week	
			Hb. G.%	PCV %	Hb G.%	PCV %	Hb G.%	PCV %	Hb G.%	PCV %	Hb G.%	PCV %
1.	B.T.	M	4	20	4.5	20	7	31	...	...	8	34
2.	B.		Less than 3 G.	14	4	18	6	23	7	30	...	...
3.	D.K.	M	4	20	...	...	5.5	24	5.5	23	6.5	30
4.	M.B.	M	4.5	20	6.5	29	...	...	9	36	9.5	38
5.	R.R.	M	7	33	7.5	35	8	38	...	...	8	37
6.	E.S.	M	6.5	25	7.5	35	...	...	9	39	10	41
7.	J.S.	M	7	28	8.5	32	9.5	35	10	35	10.5	36
8.	M.T.	M	3.5	16	4	19	4.5	20	6	25	6.5	27
9.	B.J.	M	5	29	5	29	5.5	31	6.5	33	7.5	36
10.	K.R.	M	5.5	23	6	28	6.5	30	7.5	33	8	35
11.	J.D.	M	4.5	20	5	23	5.5	24	6.5	28	7	28
12.	C.A.	M	4.5	19	5	20	6	25	6.5	26	7	28
13.	R.D.	M	4	16	4.5	18	...	...	4.5	20	4.5	22
14.	M.A.	M	5	24	5.5	26	7	30	8	33	8.5	33
15.	R.K.	F	4.5	17	5.5	22	6.5	25	...	...	7	27
16.	M.S.	M	4	16	4.5	18	5.5	23	6.5	27	8	35
17.	J.S.	M	7.5	20	7.5	20	8.5	29	9.5	32	10	34
18.	S.K.	F	3	12	5	21	6	25	...	...	6.5	28
19.	F.P.	M	4.5	17	5.5	22	7	26	...	...	...	...
20.	R.V.	M	3	11	5.5	15	6	17	6.5	20	...	...

Mean percentage Increase	1st week	2nd week	3rd week	4th week
Hb.	16.95	37.71	52.97	65.69
	S.E.±5	S.E.±10.24	S.E.±8.7	S.E.±7.52
PCV	26%	42%	52%	62%
	S.E.±39	S.E.±5.6	S.E.±8.24	S.E.±6.86

SUMMARY

A comparative study of iron carbohydrate complex and ferrous gluconate in the treatment of iron-deficiency anemia is reported. Results from animal experiments, human volunteers and clinical trial show that iron carbohydrate complex compares favourably with ferrous gluconate.[1]

ACKNOWLEDGMENTS

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TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION

There have been many investigations on the effects of iron administration and their results do not always agree. In his previous paper, the author demonstrated that iron carbonate could be readily absorbed, caused marked increases in the blood iron level, and was eliminated mainly through feces, rarely through the kidneys. He also made a full report of the experiment to the effect that, in large-dose administration of iron, once specific levels had been reached by various organs, additional intake of iron no longer elevated the iron levels and the iron merely passed through the intestines. Although iron amounts to only 0.004% of the total body weight under normal condition and the amount of tissue iron is extremely minute, iron contributes to the formation of hemoglobin and is an important contact substance for internal oxidation, carrying oxygen needed for the oxidation and removing carbon dioxide produced by oxidation. Iron is found in various foods in the form of inorganic or organic compound. The amount of iron retained varies according to the life habit of individual animals. The author already reported that a patient with Kaschin-Beck disease revealed large amounts of iron pigments in various organs, particularly in the spleen and liver, due to his daily intake of iron-containing water (Manchurian Medicine, Vol. 25, 1936). The effect of chronic intake of iron on the amount of organ deposit iron is not fully clear. Studies on this subject have been carried out by Forbes and Swift (1926), Peterson and Elvehjem (1927), Tanaka (1930), Okita (1934), Imura (1935), etc. However, no attempt has yet been made to study the effects of continuous and prolonged iron administration as the author recently achieved by means of chemical

quantitative determination and microscopic observation. The results of the author's experiment are hereby presented for evaluation by those interested in the subject.

CHAPTER 2. EXPERIMENTAL MATERIALS AND PROCEDURES

Mature, healthy Japanese rabbits were subjected to the experiment. The dosage and other experimental conditions and procedures were same as those in the previous experiment.

The rabbits were phlebotomized from the jugular vein and the organ blood was removed as completely as possible. Organ specimens fixed with pure alcohol were prepared for microscopic examination and, for chemical quantitative determination, organ specimens were pulverized and dried until no change change in quality and weight was indicated. The specimens fixed with pure alcohol were then stained with Berlin blue. For the quantitative determination of iron, 0.1 g of dry material was incinerated according to Neumann's procedure, and combined with water until the total quantity became 5 l, and a 1 mol potassium thiocyanate solution, 5 l, was added to the solution. Mohr's salt of 0.00002 g concentration was employed as analytical standard. The colorimetric procedure and colorimeter used were same as in the previous experiment.

CHAPTER 3. EXPERIMENTAL RESULTS

SECTION 1. CONTROL GROUP

Two rabbits were used as control animals: a male Japanese rabbit weighing 1460 g, and a female Japanese rabbit weighing 1620 g.

1.1 CHEMICAL QUANTITATIVE DETERMINATION

The results are shown in the table below.

IRON CONTENTS PER GRAM OF DRY ORGAN SPECIMEN - CONTROL GROUP

器官 a	例 c	1	2	器官 a	例 c	1	2
d 肺	臟	0.426	0.935	n 十二指腸		0.570	0.470
e 心	臟	0.711	0.101	o 空腸		0.540	0.480
f 肝	臟	3.650	2.190	p 迴腸		0.350	0.600
g 腎	臟	0.490	0.580	q 盲腸		0.320	0.460
h 脾	臟	10.000	9.000	r 虫樣突起		0.490	0.780
i 骨	髓	0.320	0.700	s 結腸上部		0.350	0.210
j 筋	肉	0.142	0.142	t 結腸中部		0.350	0.290
k 胃 大	彎	0.355	0.350	u 結腸下部		0.350	0.710
l 胃 小	彎	0.780	0.355	v 直腸		0.210	0.930
m 胃 幽門	部	0.350	0.142				

Keys: a, organ; b, iron content (mg); c, case; d, lung; e, heart; f, liver; g, kidney; h, spleen; i, bone marrow; j, muscle; k, major curvature of stomach; l, minor curvature of stomach; m, pylorus; n, duodenum; o, jejunum; p, ileum; q, cecum; r, vermiform process; s, upper colon; t, middle colon; u, lower colon; v, rectum

1.2 MICROSCOPIC FINDINGS

CASE 1.

Heart: No iron pigment was noted.
Lungs: No iron pigment was noted.
Liver: Granular or diffuse precipitation was noted in the liver cells surrounding the lobules. Some star cells indicated slight swelling. Iron reaction was positive, but the amount of iron pigments was extremely small.
Kidney: Kidney corpuscles exhibited diffuse precipitation of iron pigment, but the amount was minute.
Spleen: The medullary substance revealed granular and diffuse iron precipitation and some reticulum cells were slightly swollen and contained iron pigments, and the degree of change was slightly notable.
Muscle: Iron reaction was negative.
Bone marrow: Granular or diffuse precipitation of coarse iron pigments was shown by reticulum cells.
Stomach, Minor Curvature and Major Curvature, and Pylorus: Iron reaction was negative.
Duodenum: An extremely small amount of iron granules were found in the glandular cells and proper tunic of the upper layer of the mucous membrane.
Jejunum: There were free cells containing iron granules near the mucous membrane, particularly in the muscular layer of the membrane. Granular and diffuse precipitation was also present, but the amount of iron pigments was minute.
Ileum: The impression was similar to the above observation.
Cecum: Localized granular and diffuse precipitation was observed in the uppermost mucous membrane. Iron granules were also present to a relatively notable degree in the endothelium of the lymph vessel of the mucous membrane.
Vermiform Process: The lymphatic follicles revealed a small amount of iron pigments.
Colon: Iron reaction was negative.

CASE 2.
Heart: No iron pigment was observed.
Lung: There were free cells containing small amounts of iron granules in the alveolar wall.
Liver: Iron granules were found in the liver cells surrounding lobules, and some star cells were swollen and contained iron pigments.
Kidney: No iron pigment was observed.
Spleen: Reticulum cells revealed generally notable granular and diffuse precipitations of iron pigments.
Muscle: Iron reaction was negative.
Bone Marrow: Reticulum cells revealed granular precipitation of iron pigments.
Stomach: No iron pigment was found in any part of the stomach.
Duodenum: There were diffuse or granular precipitation of iron pigments in the proper tunic and glandular cells of the mucous membrane, but the amount of pigments was small.
Jejunum, Ileum: Same as duodenum.
Cecum: Slightly notable, localized granular or diffuse precipitation was oabserved in the glandular cells and proper tunic of the upper layer

of the mucous membrane. The inner coat of lymph vessel also indicated a considerable amount of iron granules.

Vermiform Process: The lymphatic follicles revealed diffuse iron precipitation.

Upper Segment of the Colon: Same as the cecum.

Middle Segment of the Colon: Iron granules were found in the upper layer of the mucous membrane and diffuse precipitation, in the proper tunic of the membrane, but the quantity was minute.

Lower Segment of the Colon: The uppermost layer of the mucous membrane revealed iron granules but the quantity was minute.

Colon: Iron reaction was negative.

The microscopic findings of the two cases are compared in the table below.

例a \ 器b	c 肺	d 心	e 肝	f 肾	g 脾	h 骨髓	j 肌	k 胃大弯	l 胃小弯	m 幽门	n 空肠	o 回肠	p 盲肠	q 阑尾	r 结肠	s 结肠	t 直肠	u 肌
1	−	−	+	+	++	++	−	−	−	−	+	+	+	++	+	++	+	+	−
2	+	−	+	−	++	++	−	−	−	−	+	+	+	++	+	++	+	+	−

Keys: a, case; b, organ; c, lung; d, heart; e, liver; f, kidney; g, spleen; h, bone marrow; i, muscle; j, major curvature of stomach; k, minor curvature of stomach; l, ;ylorus; m, duodenum; n, jejunum; o, ileum; p, cecum; q, vermiform process; r, upper colon; s, middle colon; t, lower colon; u, rectum.

SECTION 2. EFFECTS OF IRON ADMINISTRATION

Case 1. 1500 g; male.
Case 2. 1450 g; female
Case 3. 1315 g; female
Case 4. 1795 g; male
Case 5. 1351 g; male
Case 6. 1350 g; female
Case 7. 1600 g; female
Case 8. 1450 g, female
Case 9. 1500 g; male
Case 10. 1750 g; male

2.1 QUANTITATIVE DETERMINATION

The experimental results are shown in the following table.

TABLE. IRON CONTENTS (mg) PER GRAM OF DRY ORGAN SPECIMEN FOLLOWING CONTINUOUS ADMINISTRATION OF IRON

Keys: a, case; b, period of administration (days); c, lung; d, heart; e, liver; f, kidney; g, spleen; h, bone marrow; i, muscle; j, major curvature of stomach; k, minor curvature of stomach; l, pylorus of the stomach; m, duodenum; n, jejunum; o, ileum; p, cecum; q, vermiform process; r, upper colon; s, middle colon; t, lower colon; u, rectum

a 例	1	2	3	4	5	6	7	8	9	10
b鉄投与日数	2	3	5	8	10	15	20	25	30	35
c肺 臓	4.000	5.700	5.050	2.480	7.020	9.410	6.150	6.400	5.910	6.850
d心 臓	5.330	3.270	5.150	4.420	2.130	3.290	2.560	4.080	4.050	5.750
e肝 臓	7.110	3.300	8.000	4.050	7.810	3.250	8.090	7.110	4.220	7.110
f腎 臓	1.450	1.980	4.580	2.780	2.420	3.550	4.150	1.680	3.250	3.700
g脾 臓	11.500	9.000	21.200	15.500	14.510	20.560	32.000	18.290	13.070	13.760
h骨 髄	3.500	5.760	6.080	5.707	4.810	5.140	4.950	7.750	4.250	4.700
i筋 肉	0.240	0.511	1.210	0.213	0.420	0.420	0.790	0.800	1.210	0.950
j胃 大 彎	0.460	1.200	2.510	0.427	1.210	0.950	2.330	0.990	0.490	3.330
k胃 小 彎	0.300	1.470	3.020	0.989	1.040	1.020	1.550	0.110	0.410	1.150
l胃 幽門部	0.290	0.960	1.510	0.350	0.810	0.320	0.900	2.400	0.440	1.500
m十二指腸	0.570	0.280	1.330	0.140	0.780	0.510	0.850	3.120	0.720	1.260
n空 腸	0.610	1.260	1.980	0.210	3.070	2.300	2.150	2.550	1.410	1.090
o回 腸	0.530	0.720	0.510	0.240	1.210	0.430	0.980	1.700	1.220	1.180
p肝 臓	4.710	12.000	8.330	0.740	8.160	10.000	4.760	9.140	6.210	5.120
q膵 臓 実 質	0.550	1.110	3.700	0.290	1.090	1.470	1.980	2.070	7.110	5.150
r結 腸 上 部	0.410	1.720	4.170	2.070	4.810	8.090	4.060	4.740	4.710	8.000
s結 腸 中 部	1.940	1.450	2.320	0.740	4.100	3.090	0.150	2.000	4.210	3.450
t結 腸 下 部	1.220	0.810	0.750	0.740	0.690	1.540	0.610	1.990	1.490	2.100
u肝 臓	0.520	0.450	1.080	0.296	0.850	0.360	3.270	1.980	1.110	0.870

As compared to an average of 0.71 mg of the control, the iron level in the lung reached 4 mg in 2 days, and a peak was shown on the 15th day with a value of 9.41 mg. The quantity of iron per unit weight of dry lung specimen did not always increase with elapsed time, dropping to as little as 2.48 mg on the 8th day. After the peak which occurred on the 15th day, the iron level dropped again to an approximate 6 mg.

Heart: The average of the control was 2.92 mg. The iron test group indicated a high level of 5.33 mg on the 2nd day, and considerable fluctuations thereafter, with a minimum of 2.13 mg on the 10th day. The value on the 35th day was highest, 5.75 mg.

Liver: The average value of the control was 2.92 mg. The minimum of the test group was approximately 3.30 mg, occurring on the 3rd and 15th days. The maximum, 8.09 mg, was reached on the 20th day.

Kidney: The average value of the control was 0.53 mg. The maximum value of the test group, 4.58 mg, was shown on the 5th day and the minimum, 1.45 mg, on the 2nd day.

Spleen: The average value of the control was 9.83 mg. The administration of iron caused an increase in iron content to 11.50 mg on the 2nd day, which subsequently dropped to 9.00 mg in one day, the level being lower than that of the control. Marked increases occurred after the 5th day, with a peak of 32.00 mg on the 20th day, which was followed by gradual drop to 13.76 mg on the 35th day.

Bone Marrow: The average value of the control was 0.55 mg. The minimum value was shown by the test group on the 2nd day (3.50 mg), and a peak, 7.75 mg, on the 25th day. Variation was considerable, but no indefinite elevation was indicated.

Muscle: The control showed an average of 0.14 mg. The minimum value of the test group, 0.213 mg, occurred on the 8th day, and the maximum value, 1.21 mg, on the 5th and 30th days, indicating marked variations.

Major Curvature of the Stomach: The control average was 0.35 mg. The minimum value of the test group, 0.427 mg, was shown on the 8th day, and the maximum, 3.390 mg, on the 35th day. Marked variation was noted.

Minor Curvature of the Stomach: The control average was 0.56 mg. The minimum value, 0.29 mg, was given on the 2nd day, andthe maximum, 2.40 mg, on the 25th day.

Pylorus: The control group gave an average of 0.24 mg. The minimum value, 0.29 mg, occurred on the 2nd day and the maximum, 2.40 mg, on the 25th day.

Duodenum: The control group gave an average of 0.52 mg. The minimum value, 0.14 mg, was noted on the 8th day, and was considerably lower than the control average. The maximum value, 3.12 mg, was given on the 25th day.

Jejunum: The control group gave an average of 0.51 mg. The minimum value, 0.21 mg, occurred on the 8th day, and was lower than the average value of the control group. The maximum, 1.76 mg, was shown on the 25th day.

Ileum: The control gave an average of 0.52 mg. The lowest value, 0.26 mg, was shown on the 8th day, and the value was smaller than that of the control. The maximum value, 1.76 mg, was shown on the 25th day.

Cecum: The control gave an average of 0.39 mg. The lowest value, 0.59 mg, was noted on the 8th day, and the highest value, 7.11 mg, on the 30th day.

Vermiform Process: The control gave an average of 0.63 mg. The lowest value, 0.59 mg, which was lower than the control value, was shown on the 8th day, and the highest value, 7.11 mg, on the 30th day.

Upper Segment of the Colon: The control gave an average of 0.28 mg. The lowest value, 0.64 mg, occurred on the 2nd day, and the highest value, 8 mg, on the 15th and 30th days.

Middle Segment of the Colon: The control gave an average of 0.32 mg. The lowest value, 0.15 mg, which was lower than the average value of the control, occurred on the 20th day, and the highest value, 4.21 mg, on the 30th day.

Lower Segment of the Colon: As compared to an average of 0.53 mg of the control, the lowest value was 0.59 mg, and the highest value, 2.10 mg, occurred on the 35th day.

Rectum: The control gave an average of 0.42 mg. The lowest value was 0.48 mg, and the highest value, 3.27 mg, was shown on the 20th day.

The above results are tabulated below.

TABLE

Keys: a, organ; b, result; c, control group (mg); d, experimental group; e, minimum (mg); f, period of administration (days); g, maximum (mg); h, period of administration (days); j, heart; i, lung; k, liver; l, kidney; m, spleen; n, bone marrow; o, muscle; p, major curvature of stomach; q, minor curvature of stomach; r, pylorus; s, duodenum; t, jejunum; u, ileum; v, cecum; w, vermiform process; x, upper colon; y, middle colon; z, lower colon; z', rectum.

臟器	c 對照例 (匁)	d 鐵投與例			
		e 最小量 (瓦)	f 鐵投與日數	g 最大量 (瓦)	h 鐵投與日數
i 肺	0.710	2.480	8	9.410	15
j 心臟	0.556	2.130	10	5.750	35
k 肝	2.920	3.250	15	8.090	20
l 腎	0.530	1.450	2	4.580	5
m 脾	9.830	9.000	3	32.000	20
n 骨髓	0.550	3.590	2	7.750	25
o 筋	0.140	0.240	2	1.210	5.35
p 胃大彎	0.352	0.460	2	3.390	35
q 胃小彎	0.560	0.300	2	3.090	5
r 幽門部	0.240	0.290	2	2.400	25
s 十二指腸	0.520	0.140	8	3.120	25
t 空腸	0.510	0.210	8	3.050	10
u 廻腸	0.520	0.260	8	1.780	25
v 回腸	0.390	0.590	8	16.000	15
w 盲腸突起	0.630	0.590	8	7.110	30
x 結腸上部	0.280	0.610	2	8.000	15.35
y 結腸中部	0.320	0.150	20	4.210	30
z 結腸下部	0.530	0.590	8	2.100	35
z' 直腸	0.420	0.480	3	3.270	20

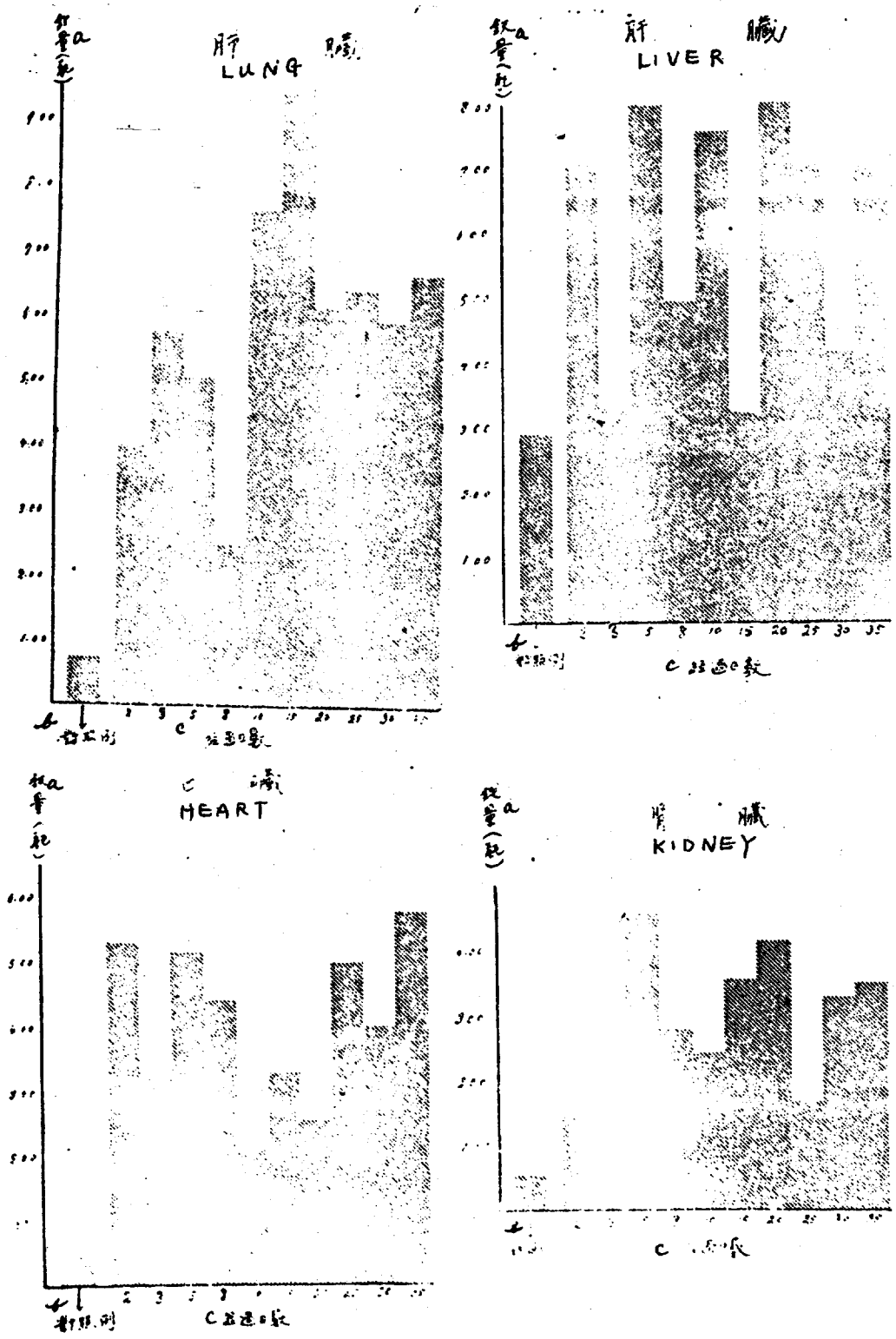
As noted on the above table, the organ iron levels failed to exhibit an indefinite pattern of elevation despite the prolonged administration of iron, and the length of the period of administration did not seem to influence the iron contents of these organs. The variation in organ iron level was considerable. The results of this experiment are illustrated in the following figures.

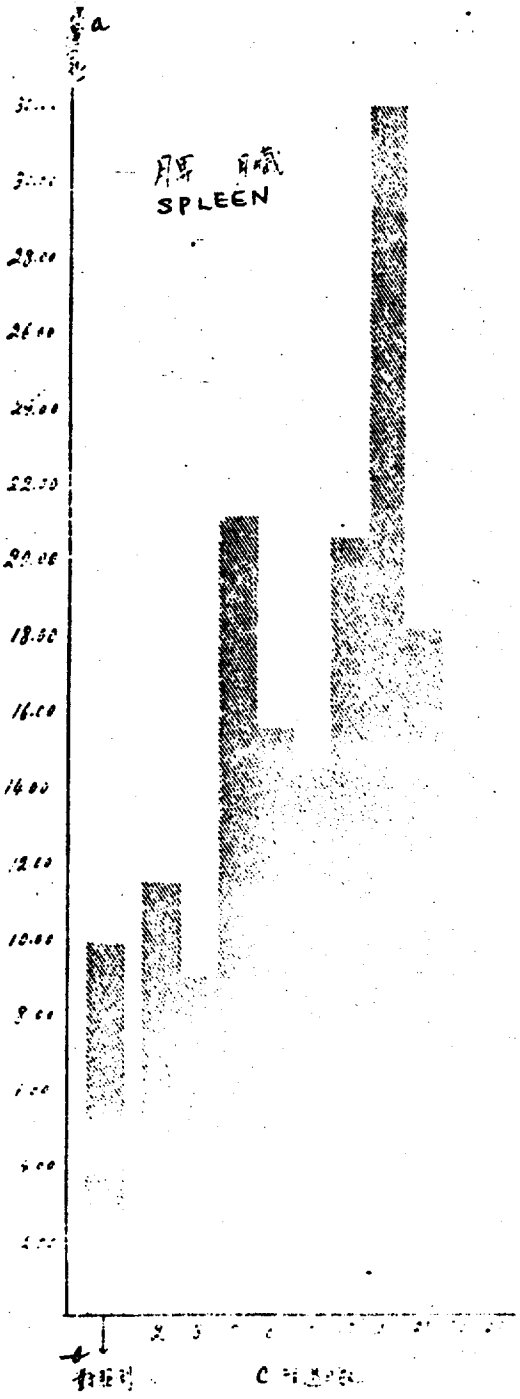
When the iron organ levels of individual cases were compared, the highest level, in terms of the deposit iron per unit weight of organ specimen, was shown by the spleen, followed in rank by the liver, lung, bone marrow, and cecum. The order of higher iron content among these organs varied according to individual cases, and no generalization cannot be made. In the alimentary tract, the cecum showed the highest level, followed in rank by the vermiform process and colon. Within the colon, the upper segment showed the highest iron level, and the level became lower toward the end. Considerable amounts of iron were also detected from the heart and kidney specimens, but muscle generally revealed only small amounts of iron.

The above discussion compared various organs in terms of iron content per unit weight of dry specimen, and the spleen showed as much as 32 mg of iron per unit weight after the experimental administration of iron. However, this particular organ exhibits a high iron level even under normal condition, as compared to other organs, and although the administration of iron elevated the level to 32 mg, the value is only 3.25 times larger than the normal value. In the liver, the normal value was 2.92 mg, and the administration of iron caused the iron level to rise to 8.09 mg. The rate of increase in this case is only 2.77 times. The following table compares various organs in terms of the rate of increase in iron content.

FIGURES

Keys: a, iron content (mg); b, control; c, period of administration (days)

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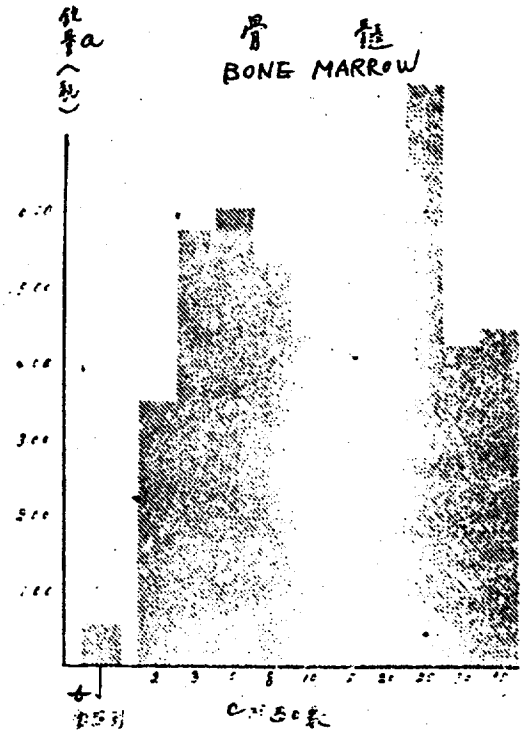
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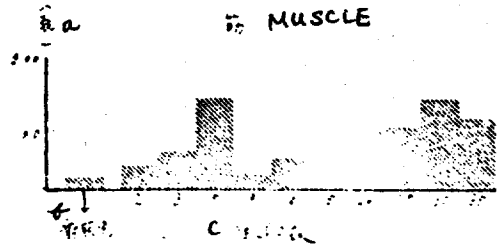
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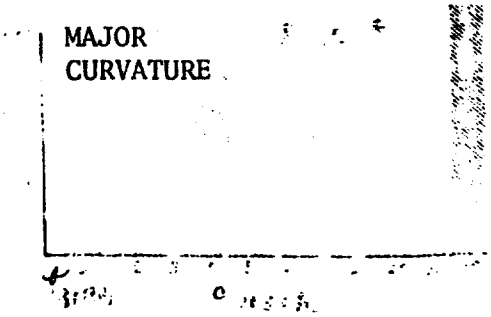
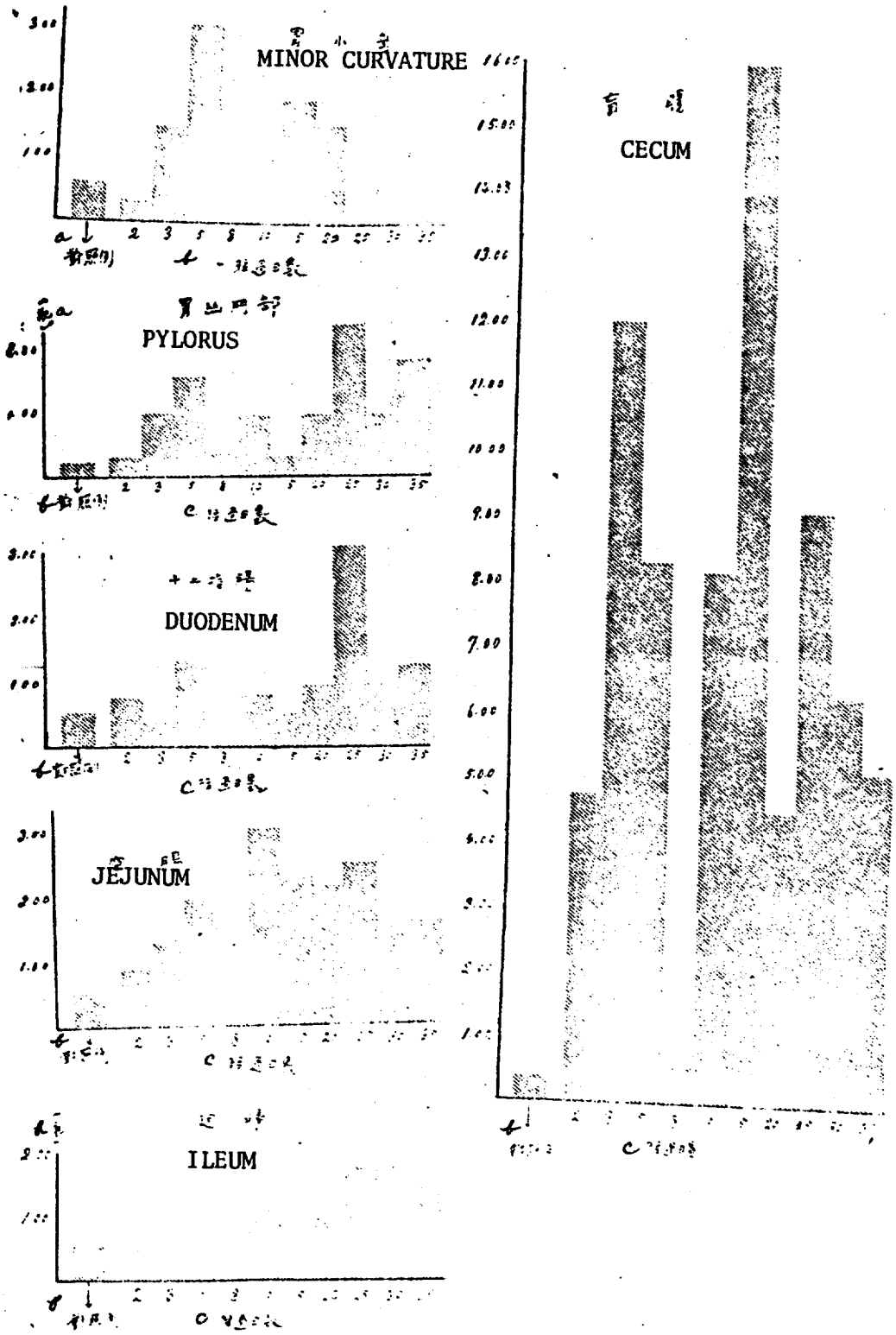

MAJOR
CURVATURE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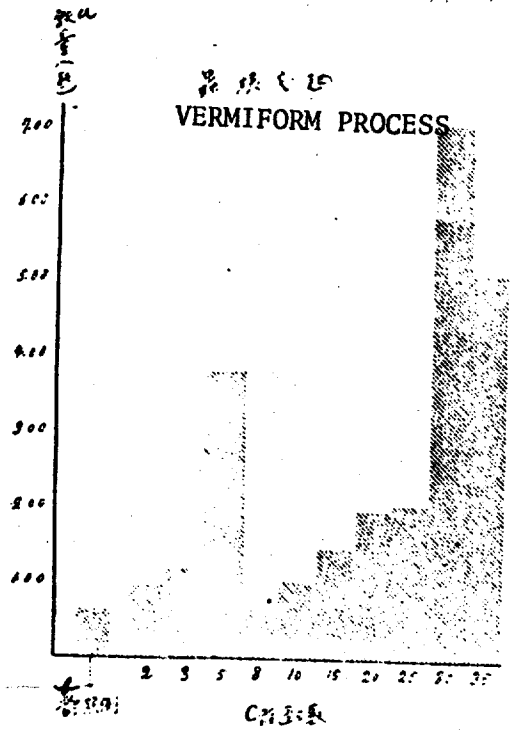
DUODENUM
JEJUNUM
ILEUM
CECUM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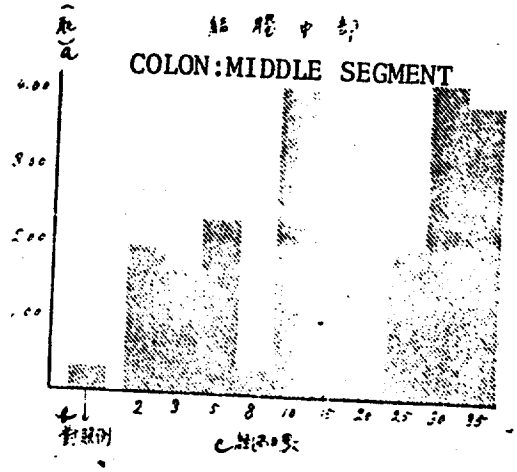
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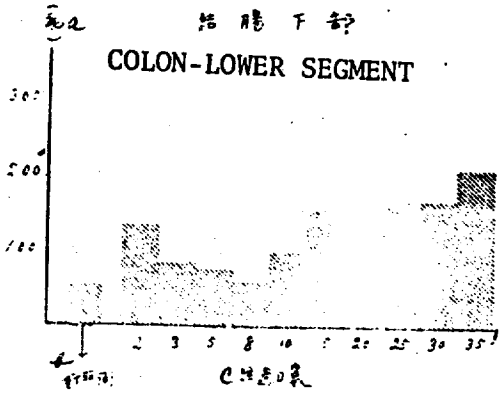
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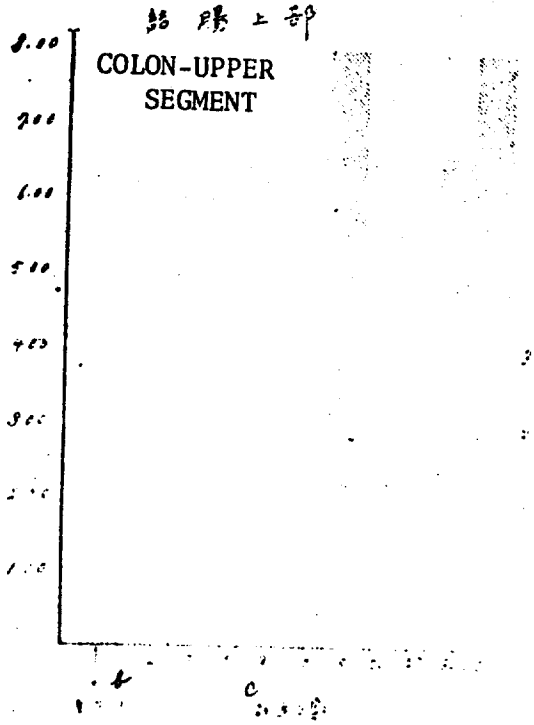
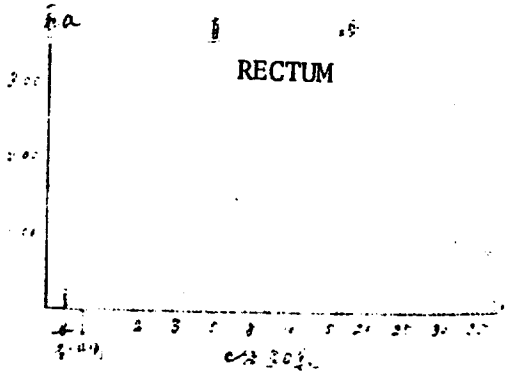

COLON-UPPER
SEGMENT


RECTUM

RATES OF INCREASE IN IRON CONTENT FOLLOWING IRON ADMINISTRATION

a 臓器名	正常鉄量 b 鏡/瓦	鉄最大量 c 鏡/瓦	増加率 d	a 臓器名	正常鉄量 b 鏡/瓦	鉄最大量 c 鏡/瓦	増加率 d
e 盲腸	0.39	16.00	41.02	o 筋肉	0.14	1.21	8.64
f 結腸上部	0.28	8.00	28.57	p 直腸	0.42	3.27	7.78
g 骨髄	0.55	7.75	14.09	q 十二指腸	0.52	3.12	6.00
h 肺臓	0.71	9.41	13.25	r 空腸	0.51	3.05	5.98
i 結腸中部	0.32	4.21	13.15	s 胃小彎	0.56	3.09	5.51
j 虫様突起	0.63	7.11	11.23	t 結腸下部	0.53	2.10	3.96
k 心臓	0.55	5.75	10.45	u 廻腸	0.52	1.76	3.38
l 胃幽門部	0.24	2.40	10.00	v 脾臓	9.83	32.00	3.25
m 胃大彎	0.35	3.39	9.68	w 肝臓	2.92	8.09	2.77
n 腎臓	0.53	4.58	8.61				

Keys: a, organ; b, normal iron content (mg/g); c, maximum iron content (mg/g); d, rate of increase; e, cecum; f, upper colon; g, bone marrow; h, lung; i, middle colon; j, vermiform process; k, heart; l, pylorus; m, major curvature of stomach; n, kidney; o, muscle; p, rectum; q, duodenum; r, jejunum; s, minor curvature of stomach; t, lower colon; u, ileum; v, spleen; w, liver.

Taking the cecum as an example, its highest iron level was 16 mg, which is considerably lower than that of the spleen, but when compared with its normal value, 0.39 mg, it shows an extremely high rate of increase, an increase by approximately 41 times. The organs showing high rates of increase are the upper segment of the colon (approximately 28 times), the bone marrow (14 times), and the middle segment of the colon (13 times). Even the muscle showed a value 8 times higher than its normal level. The increase rates of the spleen and liver are extremely low (approximately 3 and 2 times, respectively). Thus, the effect of iron on organ iron level can best be judged in terms of the rate of increase.

2.2 MICROSCOPIC FINDINGS

The specimens from 5 cases, Nos. 1, 3, 5, 7, and 9, were observed in the microscope, and the following findings were obtained.

a 例	b 投与日数	c 肺	d 心臓	e 肝	f 腎	g 脾	h 骨髄	i 筋	胃大彎	胃小彎	k 幽門部	l 十二指腸	m 空腸	n 廻	o 直腸	p 虫様突起	q 結腸上部	r 結腸中部	s 結腸下部	t 直腸
1	2	+	−	++	+	++	+	+	+	+	+	+	−	+	+	++	+	±	−	−
3	5	+	+	+	+	++	++	+	−	+	+	++	+	+	++	++	++	+	+	+
5	10	+	+	+	+	++	++	+	+	+	+	+	++	+	++	++	++	++	+	+
7	20	+	+	++	+	++	++	+	+	+	±	+	+	+	++	+	++	++	++	+
9	30	++	+	++	+	++	++	+	+	+	+	+	++	++	++	++	++	±	+	+

Keys: a, case; b, period of administration (days); c, lung; d, heart; e, liver; f, kidney; g, spleen; h, bone marrow; i, muscle; i, major curvature of stomach; l, minor curvature of stomach; l, pylorus; m, duodenum; n, jejunum; o, ileum; p, cecum; q, vermiform process; r, upper colon; s, middle colon; t, lower colon; u, rectum.

Histological examination revealed no sign of indefinite increase in iron organ level due to the administration of iron, and the iron in the tissues did not always increase with longer period of administration.

Lung: Granular precipitation of iron pigments was noted in the alveolar walls, but the amount of pigments was generally small.

Heart: An extremely small amount of iron pigments was detected among muscular fibers.

Liver: Iron color reaction was positive in the liver cells and their nuclei. Granular or diffuse precipitation appeared from the perilobular region toward the center. Star cells were notably swollen, and contained iron pigments. Iron pigments were also found in the interstices.

Kidney: Extremely minute quantities of iron pigments were found in the epithelial cells of Henle's canals, kidney corpuscles and the main portion.

Spleen: The reticulum cells indicated marked swelling and notable increased iron pigments. The splenic pulp revealed pronounced granular or diffuse precipitation of iron pigments, its iron retention being most notable among various organs.

Bone Marrow: Large amounts of iron pigments were found in the reticulum cells and the inner coat of the blood vessels.

Muscle: Iron pigments were found among muscular fibers but the amount was extremely minute.

Stomach: Generally, the amount of iron pigments was small.

Duodenum, Jejunum, and Ileum: Granular or diffuse precipitation of iron pigments was observed in the glandular cells and the proper tunic, but the amount was generally small.

Cecum: The administration of iron increased iron pigments to a notable degree. Large amounts of iron pigments in granular or diffuse distribution were found in the portion of the mucous epithelium which is in contact with the intestinal lumen. The proper tunic also indicated iron pigments, but not to any significant degree. The endothelial cells of the lymph vessels were swollen with iron pigments, and the lymph vessels and the surrounding areas revealed cells containing iron granules.

Vermiform Process: Iron pigments were found in the lymphatic follicles. The manner of precipitation in the proper tunic and the glandular cells of the mucous membrane resembled that in the cecum, but the quantity was somewhat smaller.

Colon: The pattern of precipitation was generally similar to that of the cecum, but the amount of pigments decreased toward the lower portion.

Rectum: Extremely minute amounts of iron pigments were observed in the proper tunic and glandular cells.

Summarizing the above findings, abrupt increases in iron pigments following the administration of iron were shown mainly by the spleen, cecum, vermiform process, upper segment of the colon, bone marrow, and liver.

SECTION 3. THE EFFECTS OF THE WITHDRAWAL OF IRON ADMINISTRATION

A total of 11 rabbits received iron for 38 days consecutively and the administration of iron was withdrawn. Changes in organ iron level were examined.

Case 1, 2150 g, female; case 2, 2550 g, male; Case 3, 150 g, female; case 4, 2250 g, female; case 5, 2050 g, male; case 6, 1250 g, male; case 7, 1680 g, male; case 10, 2650 g, female; case 11, 1560 g, male.

3.1 QUANTITATIVE DETERMINATION

The results are summarized in the following table.

例 a	1	2	3	4	5	6	7	8	9	10	11
経過日数 b	3	5	7	10	13	16	19	22	25	28	34
肺 c	5.330	10.660	6.100	2.690	12.800	6.490	9.110	11.850	12.800	8.000	11.600
心 臓 d	2.180	7.560	5.330	4.050	3.870	3.650	4.090	3.950	6.210	8.000	6.660
肝 e	5.330	14.090	12.800	16.090	16.090	9.050	8.970	4.260	3.950	12.800	3.990
腎 f	3.650	7.610	5.510	8.150	7.210	4.920	6.720	4.650	5.910	4.560	2.050
脾 g	32.660	32.660	32.000	24.000	25.000	32.000	32.000	32.000	32.000	22.660	21.900
骨 髄 h	8.010	8.500	7.110	4.800	1.360	2.560	4.440	3.270	4.000	3.120	2.050
筋 i	1.650	1.650	1.910	1.600	0.400	0.560	0.400	0.400	1.800	0.400	0.300
胃 大 彎 j	1.760	2.200	1.840	2.200	1.610	1.050	0.990	1.420	0.460	1.200	0.850
胃 小 彎 k	1.850	1.150	1.400	1.200	0.950	0.960	2.270	1.010	1.600	0.750	0.650
胃幽門部 l	1.900	1.300	1.200	1.000	1.560	1.120	0.960	1.420	0.140	0.580	0.420
十二指腸 m	2.660	1.400	1.810	2.240	1.870	0.900	0.960	1.300	0.870	0.670	0.830
空 腸 n	0.450	1.310	1.330	0.850	0.950	0.630	0.900	0.450	0.490	0.850	0.770
廻 腸 o	0.880	1.370	1.470	1.220	0.990	1.200	0.950	0.960	0.760	0.960	0.850
盲 腸 p	3.450	16.000	10.900	3.200	5.120	6.090	8.050	7.110	9.990	8.990	16.000
虫様突起 q	1.130	2.050	1.530	1.220	0.930	0.730	0.630	1.490	0.830	0.420	0.580
結腸上部 r	5.000	12.800	7.110	4.200	2.950	5.800	3.550	3.870	2.950	4.570	3.760
結腸中部 s	1.360	2.650	7.110	0.930	1.210	1.080	1.760	0.980	1.210	1.360	0.950
結腸下部 t	2.050	1.950	3.700	2.050	3.630	1.080	1.980	0.990	0.880	0.650	0.810
直 腸 u	1.760	1.760	1.640	1.360	1.030	0.950	0.830	1.010	0.780	0.950	0.810

Keys: a, case; b, number of days; c, lung; d, heart; e, liver; f, kidney; g, spleen; h, bone marrow; i, muscle; j, major curvature of stomach; k, minor curvature of stomack; l, pylorus; m, duodenum; n, jejunum; o, ileum; p, cecum; q, vermiform process; r, upper colon; s, middle colon; t, lower colon; u, rectum

The lung and heart showed notable increases in iron content even after the administration of iron had been withdrawn, and no sign of decrease was observed.

Liver: Except for the 5.330 and 12.800 mg on the 3rd and 28th days, respectively, after the last iron administration, the iron level became lower after the withdrawal, but the value on the 34th day after the last administration was still high, 3.990 mg.

Kidney: The suspension of iron administration was followed by decreases and considerable fluctuation in iron content. However, the value as of the 34th day was 2.050 mg, extremely high as compared to the control value.

Spleen: Considerable fluctuation followed the withdrawal of iron administration, but as of the 34th day, the iron level was 21.900 mg, greatly higher than the control average.

Bone Marrow: Notable fluctuation and decrease followed the suspension of iron administration, but as of the 34th day, the iron content was 2.050 mg, an extremely high value as compared to the control value.

Muscle: Except for the 1.800 mg of the 25th day after the last administrati the iron content generally took a downward trend. The value as of the 34th day was 0.300 mg, still higher than the control average.

Stomach and Small Intestine: Marked fluctuation was noted. There was a decrease in iron content, but the value as of the 34th day was still higher than that of the control.

Cecum: The iron level dropped to 3.200 mg ten days after the last administration, but rose again on the 34th day to 16.00 mg.

Vermiform Process: The withdrawal was followed by considerable fluctuation, and the value as of the 34th day was 0.580 mg, similar to the control value.

Colon, Rectum: With marked fluctuation, there was a tendency of decrease in iron content after the administration had been suspended, but the value as of the 34th day was still considerably higher than the control average.

Summarizing the findings, no sign of decrease was shown by the lung, heart, and cecum as of the 34th day after the last administration. Despite various degrees of fluctuation and decrease, the liver, kidney, spleen, bone marrow, muscle, various parts of the stomach, small intestine, colon and rectum still indicated iron contents far greater than that of the control. The iron level of the vermiform process returned to normal. With the exception of the lung, heart, and cecum, it can be concluded as that, although a slight tendency of decrease is shown for a period of 34 days after the last iron administration, the normal level could not be recovered within this period.

The experimental findings are illustrated below.

2.2 MICROSCOPIC FINDINGS

Five cases, 1, 3, 5, 8, and 11, were subjected to microscopic examination and the results are tabulated below.

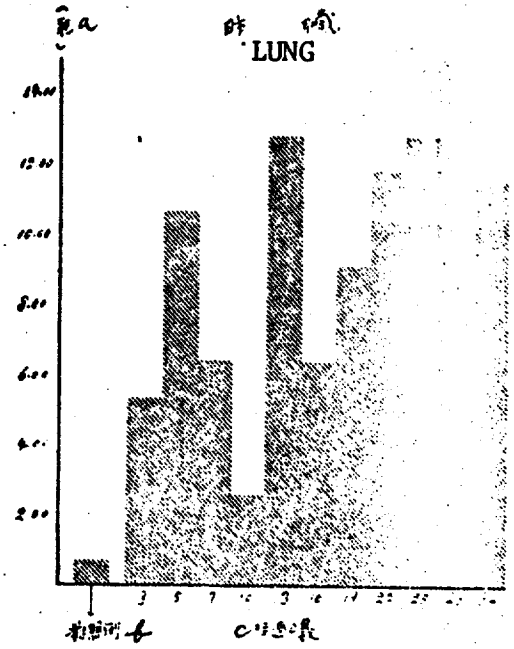
a 例	b 鉄投与中止後経過日数	c 肺	d 心臓	e 肝	f 腎	g 脾	h 骨髄	i 筋	j 胃大彎	k 胃小彎	l 胃幽門部	m 十二指腸	n 空腸	o 廻腸	p 盲腸	q 虫垂突起	r 結腸上部	s 結腸中部	t 結腸下部	u 直腸
1	3	+	+	+	+	###	##	+	+	+	+	##	+	+	###	+	###	##	+	+
3	7	+	+	+	-	###	##	-	-	-	-	+	+	-	##	+	+	+	+	+
5	13	+	+	##	+	###	##	##	+	+	+	-	+	-	###	+	##	+	+	+
8	22	+	+	+	-	###	+	+	-	+	+	+	+	+	##	+	##	+	+	+
11	34	+	+	+	+	###	##	-	+	-	+	+	+	+	##	+	+	+	+	+

Keys: a, case; b, number of days after last administration; c, lung; d, heart; e, liver; f, kidney; g, spleen; h, bone marrow; i, muscle; j, major curvature of stomach; k, minor curvature of stomack; l, pylorus; m, duodenum; n, jejunum; o, ileum; p, cecum; q, vermiform process; r, upper collon; s, middle colon; t, lower colon; u, rectum

The organ iron level indicated slight fluctuations and decreases during a period of 34 days after the last iron administration, but the pattern of iron retention in this experiment was similar to that in prolonged iron administration.

FIGURES

Keys: a, (mg); b, control; c, number of days.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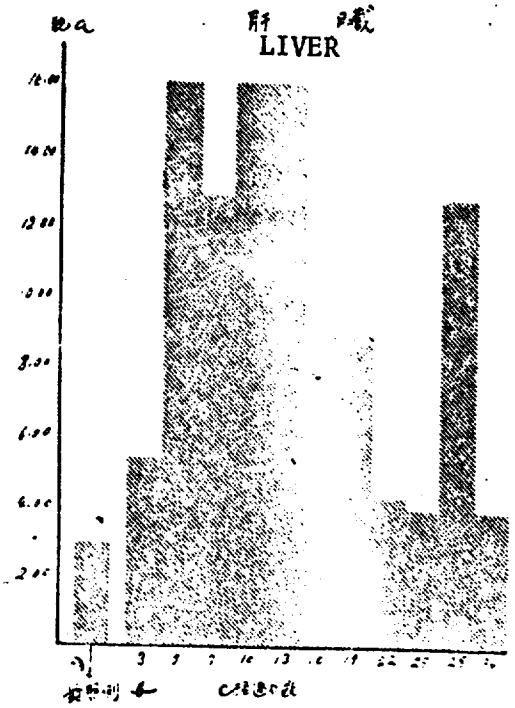
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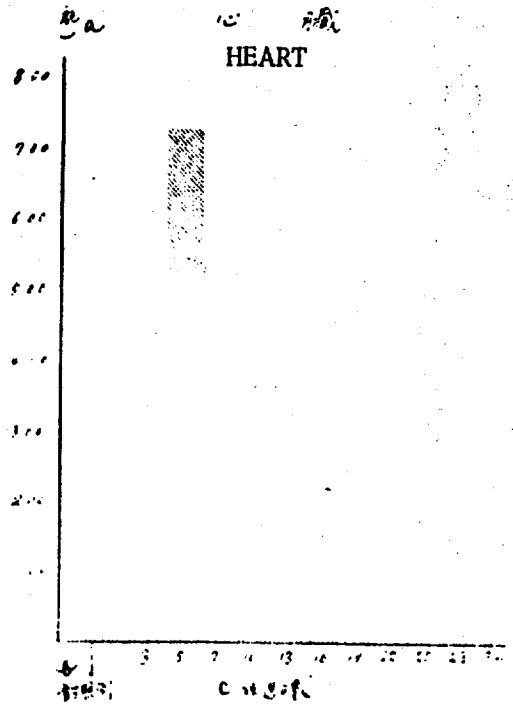
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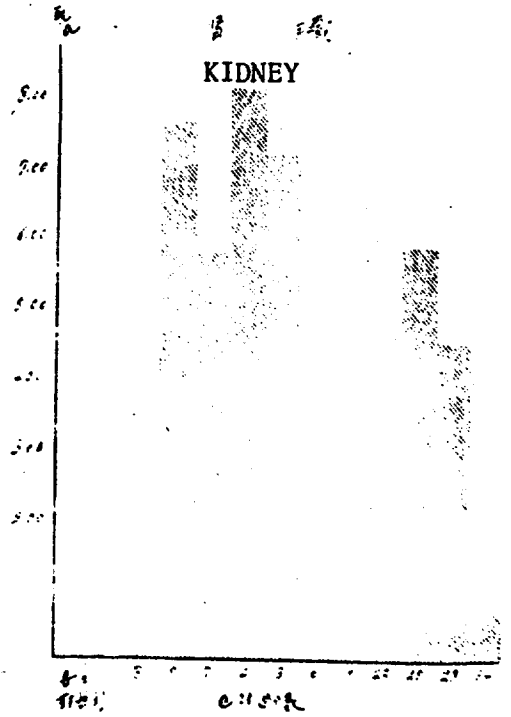
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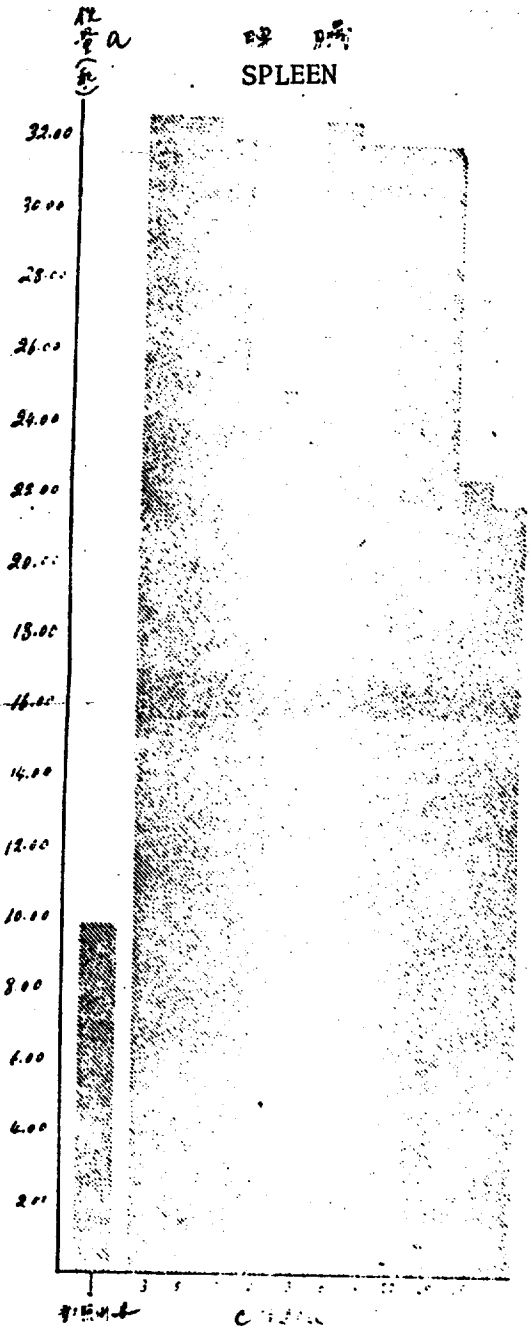
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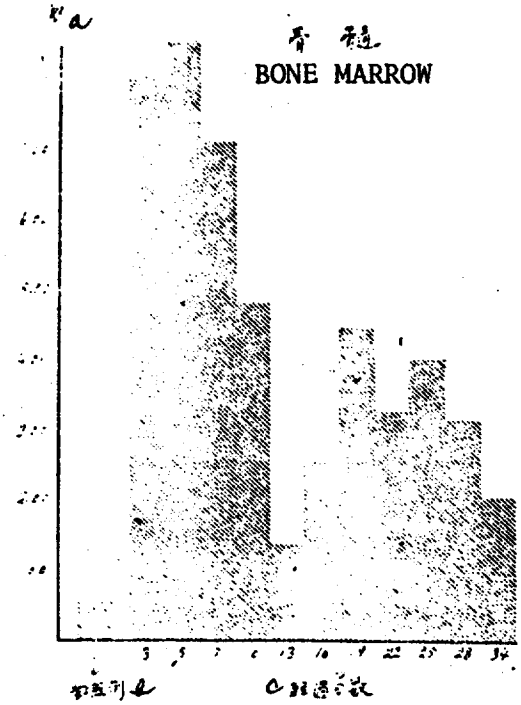
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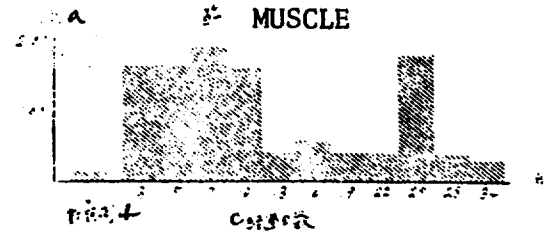
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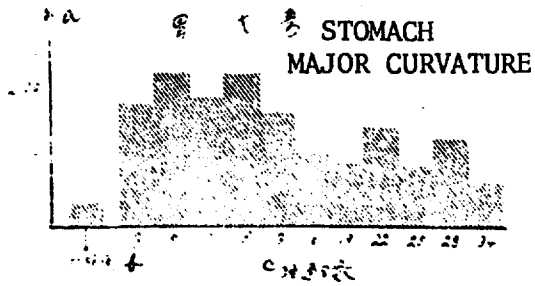
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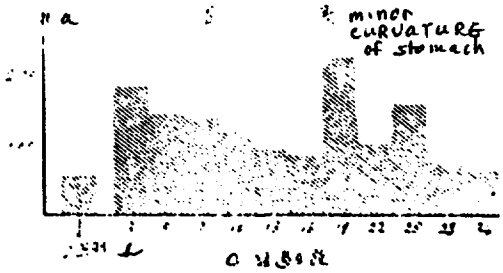
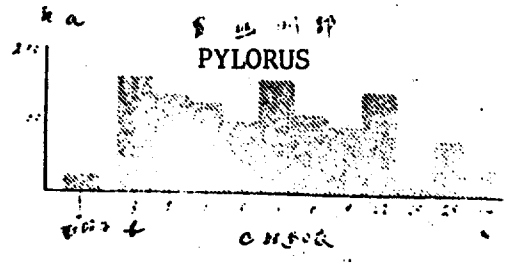
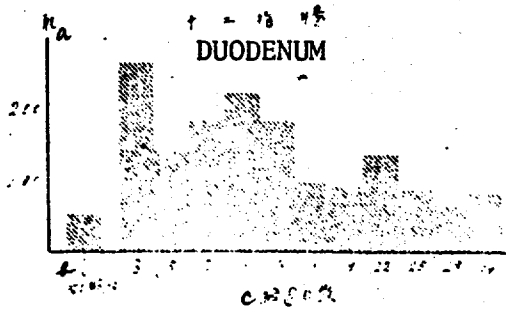

minor
curvature
of stomach


PYLORUS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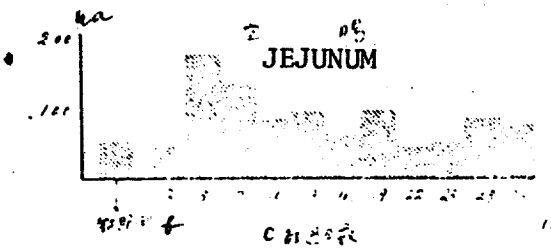
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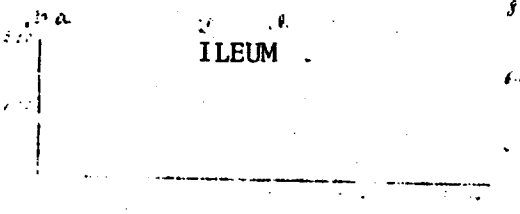
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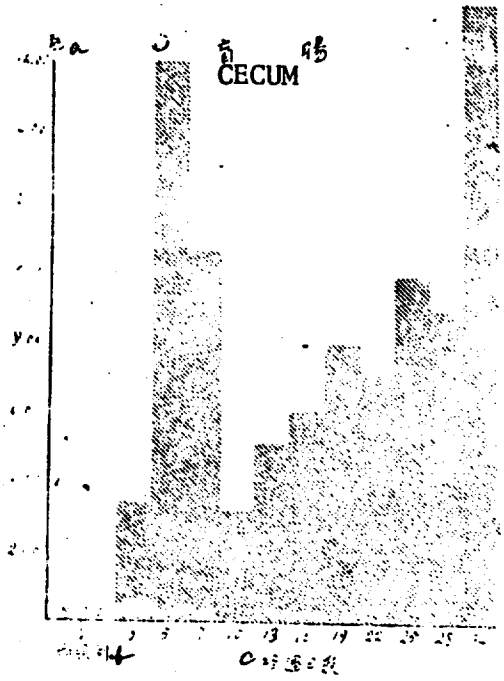
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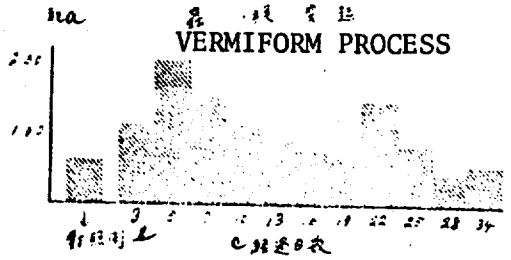
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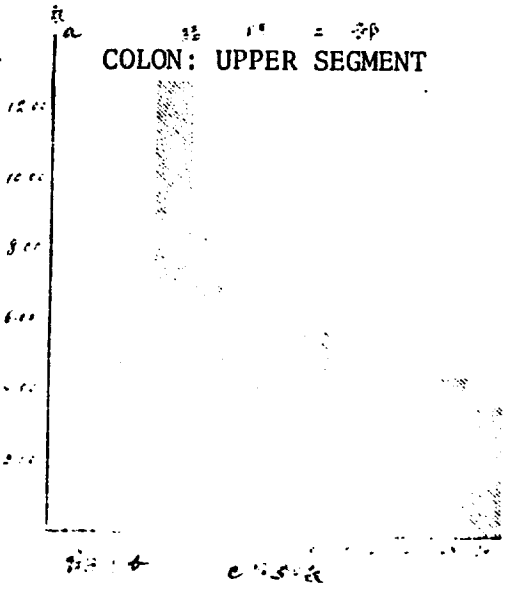
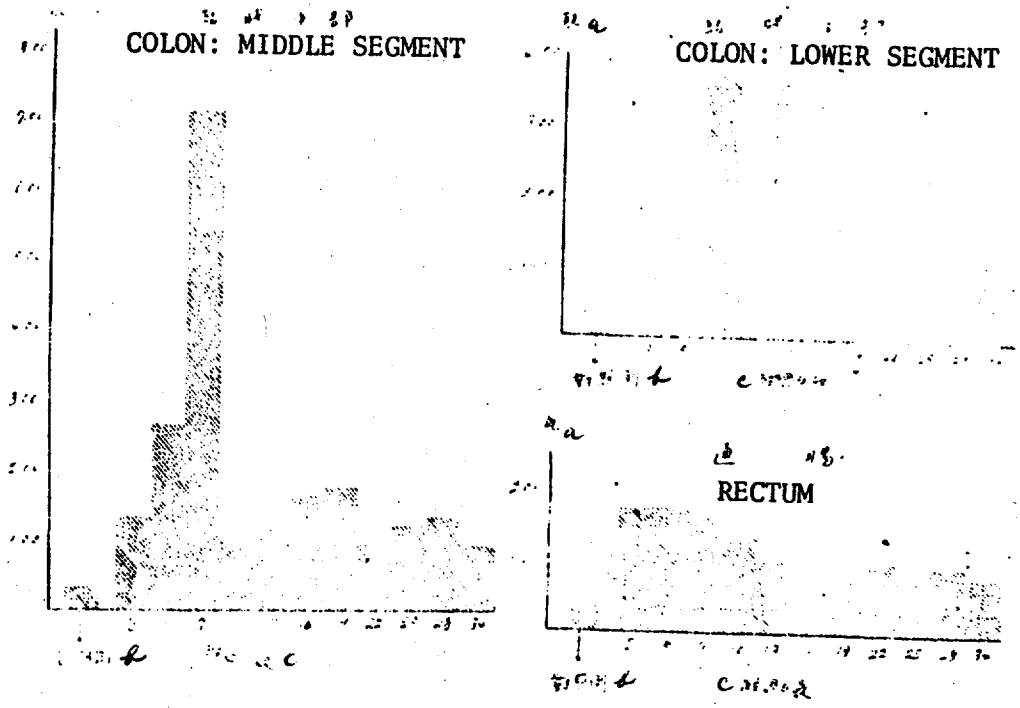
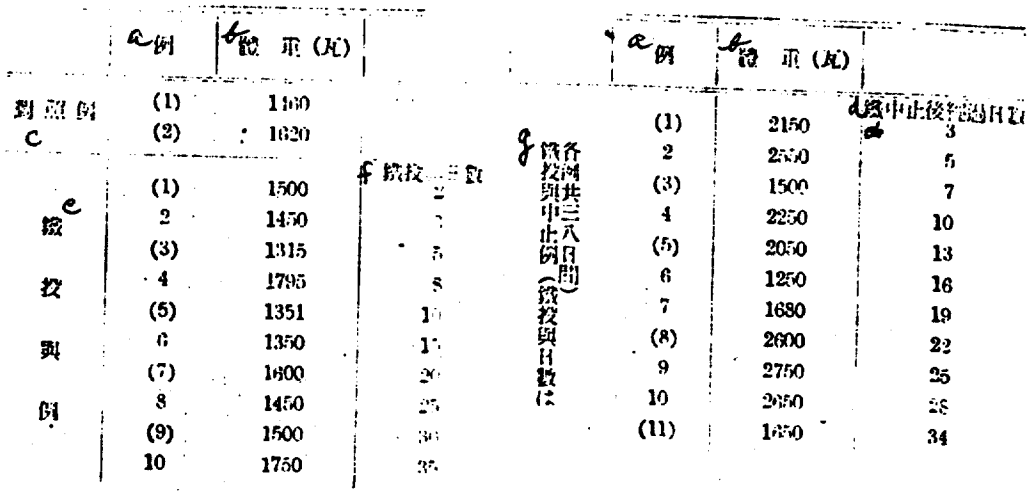

COLON: UPPER SEGMENT



CHAPTER 4. SUMMARY AND DISCUSSION

Mature, healthy rabbits were given 0.6 g of iron carbonate mixed in normal diet for prolonged periods, and changed in organ iron level were examined. The rabbits were sacrificed by phlebotomy from the jugular artery, and specimens of various organs were fixed with pure alcohol, embedded in celloidin, and stained with Berlin blue for microscopic examination. For chemical quantitative determination, other parts of the organs were dried and pulverized until their quality and quantity became stabilized, and the amount of iron per gram of specimen was measured. The organs removed for examination were the heart, lung, liver, spleen, kidney, bone marrow, muscle, all segments of the stomach, small intestine, large intestine, etc., a total of 19. The conditions and procedures of the previous experiment were followed.

Two rabbits were used as control animals, 10 were subjected to continuous and prolonged administration of iron, and 11, to iron administration for a period of 38 days. The chemical investigation was carried out on the two control animals, Nos. 1, 3, 5, 7, and 9 of the second group, and Nos. 1, 3, 5, 8, and 11 of the third group. The subjects and their body weights and experimental conditions are shown in the table below.



a 例	b 体重 (g)
対照例 c (1)	1160
(2)	1620
投与例 e (1)	1500
2	1450
(3)	1315
4	1795
(5)	1351
6	1350
(7)	1600
8	1450
(9)	1500
10	1750

a 例	b 体重 (g)	d 投与中止後経過日数
g 各例共三八日間投与例 (1)	2150	3
2	2550	5
(3)	1500	7
4	2250	10
(5)	2050	13
6	1250	16
7	1680	19
(8)	2600	22
9	2750	25
10	2650	28
(11)	1650	34

The organ specimens from the cases in parentheses were stained for iron reaction.

Keys: a, case; b, body wieght (g); c, control; e, cases subjected to iron administration; d, number of days after last iron administration; f, number of days of iron administration; g, cases subjected to iron administration for 38 days.

Let us first discuss the results of chemical quantitative determination. Some of the organs of the two control group exhibited notable individual variation, but in terms of iron concent per gram of dry specimen, the spleen indicated the highest level. The values shown by individual organs are, in the order of higher value, 9.83 mg for the spleen, 2.92 mg for the liver, 0.71 mg for the lung; 0.63 mg for the vermiform process, 0.56 mg for the minor curvature of the stomach, 0.55 mg for the heart, 0.55 mg for bone marrow, 0.53 mg for the kidney, 0.53 mg for the lower segment of the colon, 0.52 mg for the duodenum, 0.52 mg for the ileum, 0.51 mg for the jejunum, 0.42 mg for the rectum, 0.39 mg for the cecum, 0.35 mg for the major curvature of the stomach, 0.32 mg for the middle segment of the colon, 0.28 mg for the upper segment of the colon, 0.24 mg for the pylorus, and 0.14 mg for the muscle.

During a period of 35 days of iron administration, the lung showed the lowest level, 2.480 mg, on the 8th day and the highest value, 9.410 mg, on the 15th day.

The liver gave the lowest value, 3.250 mg, on the 15th day and the highest value, 8.090 mg, on the 20th day. The lowest iron content of the spleen, 9.000 mg, which is slightly lower than the control value, occurred on the 3rd day, and the highest value, 32.00 mg, on the 20th day. It was generally noted that the iron content did not always increase proportionally to the length of iron administration and the iron levels of some of the organs dropped even below the control level. The organs which showed peaks on the 35th day were the heart, muscle, major curvature of the stomach, and upper segment of the colon. Despite marked fluctuation in value, the organ iron level never rose beyond a certain limit.

Following the administration of iron, the highest iron level was shown by the spleen, followed in rank by the cecum, lung, liver and bone marrow, and the lowest level by the muscle. The highest rate of increase in iron content was given by the cecum, followed in rank by the upper segment of

the colon and bone marrow, and the lowest rate, by the spleen and liver. This indicatesthat the changes in iron content per unit weight of organ did not necessarily follow the same pattern as that of the rate of increase.

Of the group of animals which were given iron for 38 days consecutively and iron-free diet thereafter, the organs which indicated no sign of decrease in iron content during a 24-day period from the last iron administration included the lung, heart, and cecum. Specifically, the lung contained 11.850, 12.8000, and 11.600 mg of iron on the 22nd, 25th, 28th, and 34th days, respectively after the last administration, the values being considerably larger than the control value. For the heart, the values were 3.950, 6.210, 8.00, and 6.660 mg, respectively. The cecum gave 7.110, 9.990, 8.990, and 16.000 mg on the days after the 2nd day from the last administration, indicating no sign of decrease.

The liver exhibited some degree of fluctuation after the last administration, the value being 9.050 mg on the 16th day, 8.970 mg on the 19th day, 4.260 mg on the 22nd day, 3.950 mg on the 25th day, 12.800 mg on the 28th day, and 3.990 mg on the 34th day, which indicate a general tendency of decrease with the exception of the 28th day, but the value on the last day was clearly higher than the control value. Thus, it was noted that the withdrawal of iron administration was followed by slight decreases but a period of 34 days was not sufficient for the organ to regain the normal iron level. Such pattern was also shown by the kidney, spleen, bone marrow, muscle, various parts of the stomach, small intestine, colon, and rectum. The vermiform process alone gave relatively normal values, 1.490, 0.830, 0.420, and 0.580 mg after the 22nd day from the last administration, and the value on the 34th day was close to the control value.

Summarizing the above results, most of these organs generally contained less iron after the last day of iron administration except for the lung, heart, and cecum which indicated no decrease, but the content was still far greater than normal. Exceptionally sharp fluctuation or the difference among individual rabbits and a pattern of change due to other conditions will be discussed in a later section. The above experimental results generally demonstrate that the organs exhibit certain limits of iron retention, the organ iron level is unlikely to rise indefinitely even by prolonged administration of iron, and once the iron level is elevated, it is difficult to normalize it.

In the histological study of organ specimens, iron reaction was generally extremely slight in the control group, but various amounts of iron pigments could be observed in the liver, spleen, bone marrow, small intestine, large intestine, particularly the cecum and the upper part of the colon, etc. In the liver, trace amounts of iron pigments were present in the perilobular liver cells and their nuclei, and swollen star cells. The spleen displayed relatively strong iron reaction, and revealed diffuse or granular precipitation of iron pigments in the medullary substance and some of swollen reticulum cells. The small intestine exhibited weak iron reaction, but localized diffuse or granular precipitation was present. in the epithelium. Parts of the cecum and the colon indicated relatively strong iron reaction, and the pattern of iron precipitation was different from that of the small intestine in that extremely localized granular or diffuse iron precipitation occurred near the free margin of the epithelium, as if the iron pigments were in the process of being released into the intestinal lumen. It was also noted that the amount of iron pigments was smaller toward the lower part of the colon. Iron pigments were also found in

the endothelium of the lymph vessel of the cecum. The vermiform process revealed iron pigments but the amount was smaller than that in the cecum. There were free cells containing minute amounts of iron granules in the lymphatic follicles. Relatively strong iron reaction was exhibited by the bone marrow or its reticulum cells.

Rabbits 1, 3, 5, 7, and 9 which received iron for 2, 5, 10, 20, and 30 days, respectively, were chosen for histological investigation. There was no tendency of indefinite increase in the amount of iron pigments with time, but slight quantitative changes could be observed. The precipitation of iron was most notable in the spleen, followed in rank by the liver, bone marrow, cecum, vermiform process, and colon. The lung, heart, kidney, muscle, stomach, small intestine, and rectum revealed minimal precipitation. The lung remained without appreciable difference from the control, but the heart revealed iron pigments among muscular fibers. The star cells generally indicated pronounced swelling and iron pigments appeared in the star cells, liver cells, the interstices, and capillary endothelium, but, as stated above, the amount of iron pigments did not increase proportionally to the period of administration. The kidneys indicated no significant change but some iron pigments appeared in the epithelial cells of Henle's canals, kidney corpuscles and the main part. The spleen showed no appreciable difference in the pattern of iron precipitation from the control, but the amount of pigments had clearly been increased, and the reticulum cells were notably swollen. The amount of iron pigments in the spleen remained at the same level throughout the period of observation, showing no sign of indefinite increase. The bone marrow revealed pronounced iron precipitation in the reticulum cells, and, unlike the control, iron pigments appeared even in the vascular endothelium. The muscle revealed a trace amount of iron pigments among muscular fibers. There were iron pigments in the glandular cells and the proper tunic of the stomach and various parts of the small intestine, but the amount of precipitation exhibited no specific pattern. There was a clear increase in the amount of iron pigments in the cecum, and, although the pattern of precipitation indicated no appreciable difference from the control, the endothelium of the lymph vessel in the mucous membrane was swollen with deposit iron, and cells containing iron granules were frequently found near the lymph vessel. The amount of precipitation in the cecum also failed to exhibit a pattern of indefinite increase. The condition of iron precipitation in the vermiform process or colon resembled that of the cecum, but a quantitative decrease was definitely indicated, particularly in the lower segment of the colon. Extremely minute amounts of iron pigments were found in the glandular cells and proper tunic of the rectum.

As stated above, the administration of iron generally increased the amount of pigments in the liver, spleen, cecum, vermiform process and colon, but the increase was minimal in other organs. The amount did not always show an increase proportional to thellength of iron administration, the increase being confined within a specific limit. Quantitatively, there were relatively notable fluctuations.

Of a group of rabbits which were used for the observation of the effect of the withdrawal of iron administration, 5, Nos. 1, 3, 5, 8, and 11, were subjected to histological observation on 3, 7, 13, 22, and 34 days after the last administration, respectively.

The pattern of iron precipitation generally resembled that of the previously discussed group, with notable precipitation in the liver, spleen, bone marrow, cecum and colon, and minimal precipitation in other organs.

Neither the case examined on the 3rd day nor No. 11, which was examined on the 34th day, indicated no marked change in the amount or pattern of iron precipitation. It was noted that the amount of iron pigments hardly showed a decrease proportional to the length of period after the last iron administration. It was thus assuamed that the tendency to retain internally stored iron was extremely strong.

Summarizing the histological findings, the amounts of iron pigments stored in the liver, spleen, cecum, bone marrow, colon, and vermiform process showed clear increases due to the experimental administration of iron, but the increases in other organs were not significant. The amount of iron pigments followed no specific pattern of fluctuation, and the withdrawal of iron administration did not result in any sign of reduction in deposit iron. However, chemical quantitative determination of tissue iron revealed a slightly downward tendency over a period of 34 days, with the exception of a few organs, the findings being generally in agreement with the results of microscopic examination.

Thus, the histological findings on the experimental groups are clearly different from those of the control group. According to Iwao (1926), the amount of iron pigments in rabbit organs which permits their microscopic observation varies to such a notable degree even among apparently healthy rabbits that, in the study of experimental siderosis, the accuracy of the experimental findings is often questioned. In his experiment, he divided the rabbits into 3 groups according to the severity of siderosis: group 1 with minimal iron precipitation in the liver cells; group 2 with large amounts of iron pigments in liver cells; group 3 with severe iron precipitation. The control group in the author's experiment showed small amounts of iron pigments in liver cells around the lobules. Iwao described the areas showing a strong tendency to accumulate iron pigments as liver cells and star cells, reticulum cells in the bone marrow and spleen, mucous epithelia and endothelium of the lymph vessel in the mucous membrane of the intestine, the lymph gland of the mesentery, particularly the lymph glands of the cecum and the portal region of the liver, renal duct, and Henle's canals. He stated that these areas were susceptible to spontaneous siderosis, and there was a danger of spontaneous siderosis to be confused with experimental siderosis. He also denied the validity of a theory that the areas where iron was accumulated as a result of experimental iron administration must be the areas susceptible to spontaneous accumulation of iron in rabbit. According to Iwao, the precipitation of iron pigments is particularly severe in the capillary endothelia in the liver or bone marrow in experimental siderosis in rabbit, but never in spontaneous siderosis. Moreover, he observed that the small intestine exhibited marked difference from the cecum in the mode of iron reaction in mucous epithelia. He stated that the iron reaction in the mucous membrane of the small intestine was generally weak and diffuse whereas in the cecum, it occurred mainly in the uppermost portion of the epithelium, with clear localized accumulation of iron pigments. This observation demonstrates that the absorption of iron by the intestine is relatively proportional to time whereas in the cecum, iron is excreted into the intestinal lumen at specific intervals, which is substantiated by the author;s experimental results.

In the present experiment, the quantitative determination and microscopic examination did not yield the same findings on iron organ level. The chemical quantitative determination revealed extremely high iron levels in individual organs whereas the microscopic findings showed no marked increase in the amount of iron pigments in some organs. For instance, the amounts of iron in the lung, heart and cecum of the third group

indicated fluctuations after the last iron administration but did not necessarily showed decreases with time. In some cases, even increases were noted. On the contrary, the microscopic examination revealed iron precipitation in the lung and heart to be without appreciable change and that in the cecum to be without increase. Such difference was also noted in other organs. The difference may be attributed in part to insufficient phlebotomy. In the quantitative determination of the iron contained in the organs, the amount of blood remaining in the animal varies whether the animal was killed by phlebotomy or by complete vascular irrigation by means of physiological saline solution. This may also influence the organ iron level. Reviewing the opinions of other investigators in this respect, Tanaka (1930) observed in his experiment using dogs that the amount of iron contained in various organs varied slightly according to whether or not the animals were subjected to irrigation, but the difference was minimized when phlebotomy was complete. Okita (1934) regarded vascular irrigation as essential to the measurement of iron organ level. The author demonstrated in the previous paper that the blood iron level showed marked fluctuations and increases as a result of iron administration, but if the blood was removed incompletely, the remaining blood might have contributed to the high levels. Thus, it is assumed that the lack of vascular irrigation caused the marked fluctuation in the results of quantitative determination. Secondly, the difference between individual rabbits should also be taken into consideration. As is noted clearly in the author's previous experiment, the experimental conditions remained the same for both blood iron and the absorption and excretion of iron, but there were variations in experimental results due to individual difference. Such difference can also be expected from the present experiment on organ iron level. The possibility of spontaneous siderosis as suggested by Iwao should not be ignored. As Okita reported, the sex difference may also be a contributing factor. The seasonal factor has received considerable attention foryears. As is well known, the iron detected by microscopic examination tends to be more in spring rather than in winter. Nagano, Okamoto and Iwao have already proposed various theories on this subject. Moreover, in the present investigation, the author fixed histological specimens with 100% alcohol for iron reaction with Berlin blue. Iron chloride, iron nitrate and ferrous thiocyanate are soluble in pure alcohol. At the present, the form of the iron contained in animal tissue is unknown. Some investigators believe that iron is retained in the form of iron albuminate, and other believe that it is contained in the form of iron oxide. In any event, if the iron compound is alcohol-soluble, Berlin blue reaction of alcohol-fixed specimens is highly inadequate. Such circumstance might be present in the present experiment, contributing to the extreme inconsistency of its results. The author conducted microscopic examination primarily for the comparison of its findings with the results of quantitative determination and for the study of the changes in iron content after the withdrawal of iron administration, not for the observation of the condition in which iron pigments precipitated in experimental siderosis. Thus, the author does not wish to pursue this aspect any further.

For many years, whether iron is absorbed or not absorbed in an organic or inorganic form has been a subject of active debate, but it is beyond any doubt that inorganic iron can be absorbed and assimilated. It is generally believed that iron is converted to iron chloride by the gastric juice, bonds with proteins, and transferred to the intestine where iron is again liberated and absorbed from the intestine, subsequently bonding with hemoglobin and transferred to various tissues throughout the body for assimilation and utilization. However, the mechanism of its internal

absorption and the route of transfer after absorption is not fully clarified.

A marked increase in general iron content following oral intake of iron has been demonstrated by Hall (1891 - 1914), but the specific sites of increase are yet undetermined. Schmidt (1912 - 1914) maintained that dietary iron was deposited mainly in the liver and the iron due to cellular destruction, in the spleen. Nagano (1924), Horita (1926), Kunkel (1891), Woltering (1895), etc. shared the same opinion. On the other hand, Gaule (1896) held that orally ingested iron did not enter the portal system but was introduced into the blood by way of the thoracic lymph vessels and deposited in the spleen, but his assumption was opposed by Müller (1900). Abderhalden (1900) maintained that orally introduced iron appeared in the spleen and liver, and Hall demonstrated in his experiment with rats that the iron level in the spleen rose one week after the administration of iron, and that in the liver subsequently upon which the spleen level returned to normal. It is difficult to determine which of these experimental results is most valid, but in the author's experiment and Tanaka's experiment with dogs (1930), the iron content per specific amount of spleen specimen was far greater than that of liver specimen. As stated previously, the autho does not intend to pursue the route of internal transfer of iron without further investigation.

Most of the studies on organ iron level have been done by microscopic examination, rarely by chemical quantitative determination. Especially, the systematic and quantitative determination of iron content in a large number of organs, as attempted by the author, has never been carried out. The range in which the iron reaction currently performed for such purpose can be observed in the microscope is influenced by the quantitative or qualitative level of tissue iron. According to Hueck (1912), positive iron reaction can be obtained only when the amount of iron per 100 g of dry human liver specimen exceeds 500 mg. Nagano set the minimum iron level for microscopic observation of iron reaction at 53 mg for rabbit. Abderhalden and Schmidt maintained that only specific types of iron exhibited positive reaction. Thus, this type of study by microscopic observation of chemical iron reaction risks quantitative errors, and quantitative determination seems to be a more adequate method. This method was employed by Bar (1924), Forbes-Swift (1926), Peterson-Elvehjem (1927), Tanaka (dogs; 1930), Kaimei (mice), Okita (rabbits; 1930), and Imura (rabbits; 1931). Their experimental results are compared in the following table.

As noted on the table, there are marked differences in findings among these experiments, but this is due to the difference in experimental animals, conditions, and procedures. In view of the fact that the present experiment was designed to study changes in organ iron level following the administration of iron and to compare the changes with the control under specific conditions, the quantitative difference from the results of other investigations is of no great significance in regard to the purpose of this experiment. Despite the variation, these data demonstrate that the liver andspleen take part in the retention of iron, and the intestinal organs and kidneys, in iron metabolism as absorbing and excreting organs.

The failutre for the iron retained in the system to diminish even after the suspension of iron adminstration has already been reported in the previous paper. Iron is a component always present in the body cells and, as demonstrated by many investigators, cellular destruction due to a certain change in the body causes part of it to escape. Muller observed the excretion of 7 to 8 mg of iron per day by a starving man due to the fatigue of body cells. It is a characteristic of a living body to try to retain the iron in the destructed cells. Schmidt calculated the amount

of iron released upon physiological destruction of etythrocytes in a healthy adult as 60 - 100 mg a day. However, the amount actually excreted is only 1/10 of that amount. Comparing this 60 - 100 mg with the amount supposedly excreted in Muller's starvation test, at least 50 - 90 mg must have been retained during the intermediate metabolism. Thus, these investigators recognized the tendency of a living organism to persistently adhere to iron. The tendency to retain iron is ascribed to the reticulo-endothelial system which was regarded as an iron metabolism control system by Aschoff (1924), and it is readily assumed that the spleen plays the principal role in this function. The high iron level in the spleen has been observed by Nasse (1893), Kruger (1898), and Seemann (1904), Based on the fact that the amount of iron excretion increases suddenly after splenectomy, and other experimental results, Asher (1911) and his student Grossenbacher (1909), Zimmermann (1909), Vogel (1912), Sollberger (1913), Nakayama (1924), and Tominaga (1925) concluded that the spleen was the organ which controlled iron metabolism. Incorporating this view with the opinions of Roth (1912), Austin-Pearce (1914), Chevallier (1914), Schmidt (1914), Jerger (1926), Goldschmidt-Pepper-Pearee (1915), Pepper-Austin (1916), Pearel-Krumbhaar-Frazier (1917), and Horiuchi (1926) who expressed negative or affirmative stand toward Acher's assertion, it is beyond any doubt that the spleen and iron metabolism are somehow related.

ORGAN IRON LEVELS IN NORMAL RABBIT

	Peterson Elvehjem (%)	l 大北 (%)	m 井村 (mg/g)	n 林 (mg/g)
a 心臓	0.0464	0.0331	0.181 – 0.181	0.556
b 肺	0.0338	0.0482		0.710
c 肝	0.0475	0.0567	1.054 – 1.095	2.920
d 腎	0.0289		0.0805 – 0.101	0.530
e 脾	0.1565	0.1507	0.181 – 5.770	9.830
f 筋	0.0092	0.1409	0.017 – 0.036	0.112
g 骨髄	0.0206 (骨)o	0.0103 (骨)o	0.142 – 0.319	0.550
h 胃	0.0307	0.0213		0.352 (大彎部)t 0.560 (小彎部)u 0.240 (幽門部)v
i 小腸	0.0171 (腸)p	0.0318 (腸)p	0.155 – 0.090 (十二指腸)q 0.133 – 0.09 (小腸上部)r 0.121 – 0.09 (小腸下部)s	0.520 (十二指腸)q 0.510 (空腸)、 0.520 (廻腸)、
j 盲腸			0.299 – 0.329	0.390
k 大腸			0.033 – 0.088	0.630 (横行結腸中部)w 0.280 (横行結腸上部)x 0.320 (横行結腸中部)y 0.530 (横行結腸下部)z 0.420 (直腸)z'

Keys: a, heart; b, lung; c, liver; d, kidney; e, spleen; f, muscle; g, bone marrow; h, stomach; i, small intestine; j, cecum; k, large intestine; l, Okita; m, Imura (mg/g); n, Hayashi (mg/g); o, (bone); p, (intestine); q, (duodenum); r, (upper small intestine); s, (lower small intestine); t, major curvature); u, (minor curvature); v, (pylorus); v', (jejunum); v", (ileum); w, (vermiform process); x, (upper segment of transverse colon); y, (middle part of transverse colon); z, (lower part of transverse colon); z', (rectum)

After the spleen is removed, iron metabolism and other splenic functions are believed to be performed by other reticulo-endothelial system, particularly that of the liver. According to Aschoff, iron metabolism is not monopolized by the spleen, but is performed widely by all reticulo-endothelial systems, the spleen being one of the principal sites, and the elevation of liver iron level upon splenectomy is due to the compensatory activity of its reticuloendothelial system.

Various attempts have been made to classify organ iron by function. Schmidt provided two categories: functional iron (Funktiouseisen) and stored iron (Reserreisen). The former displays no positive iron reaction in the microscope (this view was opposed by Hueck and Nagano), and the latter gives a positive reaction, is derived from dietary iron and iron released by cellular destruction, and is stored in the liver, spleen, and bone marrow. Thus, he held that the dietary iron was stored in the liver whereas the iron released due to cellular destruction is deposited in the spleen andbone marrow. Kunkel and Woltering et al. also stated that dietary iron is stored in the liver for future utilization. On the other hand, Gaule maintained that dietary iron was retained in the spleen, not in the liver, and Abderhalden regarded the liver, spleen, and mesentric lymph gland as the storage of dietary iron, emphasizing the liver among them. Hall observed that the elevation of iron content in the spleen preceded that in the liver. Tanaka and Okita reportedly found a large amount of deposit iron in the spleen rather than in the liver. Nagano and Horiuchi agreed to Schmidt's view on the precipitation of iron due to cellular destruction and dietary intake.

The significance of the intestines in iron metabolism is clearly of equal importance to the role played by the liver and spleen. Orally introduced iron is excreted in a large amount into the large intestine, particularly in the cecum, as reported by MacCallum (1894), Hochhaus-Quincke (1896), Hoffmann (1898), Munk (1902), and Chevallier (1914), and this has been an established fact. Imura held the following theory. Physiologically, the cecum contains a large amount of iron, and this should not be attributed solely to its excretory function. The cecum exhibits an inherent tendency to retain iron and is closely related to the storage and absorption of iron by the liver and spleen. Thus, the deposit iron in the cecal wall increases notably following experimental administration of iron.

It is pertinent to determine if the iron in the small intestine is in the process of being absorbed or excreted. Many investigators have observed that orally introduced iron is absorbed from the upper portion of the small intestine (Cloetta, 1895; Nathan, 1900), particularly from the duodenum (Hochhaus-Quincke, MacCallum, Hall, Hoffmann, Gaule). The above observation was based on the fact that, in laboratory animals, the iron granules with positive reaction to Berlin blue or ammonium sulfide appeared only in the epithelial cells or proper tunics of the duodenum and adjoining upper part of the jejunum, not in any of the lower intestine. Hall interpreted this as that iron could not be absorbed from the organs past the duodenum since hydrogen sulfide in the intestine changed iron into a compound which could not be absorbed. In the present experiment, however, iron granules were found in the small intestine in various amounts. Contrary to the opinion held by these investigators that, in iron metabolism, the upper portion of the small intestine is significant only for the absorption of iron, Abderhalden (1909) observed that it took part in the excretion of iron as well. Chevallier (1914) demonstrated that the iron injected into a blood vessel was excreted in large amounts from the

duodenum and upper part of the jejunum as well as the cecum and large intestine. In his microscopic observation, Sawai of Japan (1926) confirmed that iron was excreted mainly from the cecum and large intestine, and occasionally from the duodenum. Tanaka and Imura also believed that iron was excreted from the small intestine. At this point, a question is raised whether the iron found in the upper small intestine is in the process of being absorbed or excreted. It is difficult to provide an answer to this question. The author observed deposit iron in the upper intestine, but whether it was on the way to be excreted or absorbed could not be determined.

As is generally known, the kidney is an excretory organ of iron and the amount found in the kidney is extremely minute. This was demonstrated in the previous part of this paper. Although histological findings alone are unable to determine whether iron pigments found in the kidney are in the process of absorption or excretion, the increase in urine iron level following iron administration seems to speak clearly for the fact that the kidney is an excretory organ of iron.

The organs so far discussed are clearly related to iron metabolism in one way or another, i.e., the liver and spleen are iron storage organs, various parts of intestines and kidney take part in the absorption and excretion of iron, but the significance of skeletal muscle in iron metabolism still remains to be determined. Okita et al. demonstrated clear elevation of muscular iron level following the administration of iron. The author also believes that muscles play some role in iron metabolism, based on the results of the pre ent experiment.

From the results summarized above, the author concludes that the measurement of organ iron level must be based on quantitative determination, the organ iron level rises to a notable degree upon iron administration but the increases are within a certain limit, and the iron once stored in the body does not diminish rapidly even after the withdrawal of iron administration. These findings have clarified some problems related to inorganic iron metabolism.

CHAPTER 5. CONCLUSION

The author studied the changes in organ iron level in normal rabbits following consecutive administration of iron carbonate at daily dosage of 0.6 g by chemical quantitative determination and microscopic observation.

1. A total of 10 rabbits received iron for a maximum period of 35 days. Following the administration of iron, the iron content per unit weight of organ showed clear increases. Despite notable variation among individual rabbits, the highest iron level was shown by the spleen, followed in rank by the liver, cecum, bone marrow, vermiform process, upper part of colon, lungs, etc. The amount of iron did not show any effect of the period of administration and remained within a certain limit. The rate of increase in deposit iron per unit weight of organ was highest in the cecum, followed in rank by the upper part of the colon and bone marrow. The lowest rates were shown by the liver and spleen. The changes in organ iron level did not agree with the rate of increase.

2. A total of 11 cases received iron and the administration of iron was withdrawn. The iron contents of various organs decreased to varying degrees when the administration of iron was suspended, but the normal level could not be attained within a period of 34 days after the last iron administration.

3. The amount of iron pigments showed sharp increases in the spleen, cecum, colon, bone marrow, and liver following the administration of iron, and normal levels could not be recovered within a period of 34 days after the last iron administration.

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ORAL ADMINISTRATION OF IRON IN HYPOCHROMIC ANEMIA

CLARK W. HEATH, M.D.
BOSTON

One of the oldest problems in which modern scientific medicine has interested itself is that concerning the efficacy of iron in the treatment of anemia. Perhaps no other problem has attracted so much thought and work with results so little in agreement. This idea has been expressed well by Whipple and Robscheit-Robbins:[1] "The history of anemia treatment with drugs is indeed a tale to make the judicious grieve."

Some of the conflicting ideas regarding iron medication may be traced to the careless application of the discoveries made in experimental anemia in animals to the anemias that occur in man. For the present, until more is known of the mechanisms involved in different types of anemia, in man and animals, the statement of Witts is much to the point, namely, that no apology is needed for considering the field of clinical medicine the testing ground of iron therapy.[2]

In the past hundred years the prescribed dose of iron in anemia has varied widely. Blaud, in 1832,[3] soon after he had announced the efficacy of certain pills which he regarded as specific in the treatment of chlorosis, voiced an objection to a formula which reduced the strength of his pills to about one-half. On the whole, not only since that time, but since at least the time of Sydenham, iron was considered of great value in the treatment of chlorosis.[4] In the hands of those physicians who found it specific in this disease it was used in large doses for prolonged periods. During that century iron was often prescribed in doses that today would be considered too small. For a period in recent years it has been relegated to a minor therapeutic position, chiefly by reason of its lack of effect experimentally in acute blood loss, possibly

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From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.

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also because of the less frequent occurence of severe chlorosis and because of its frequent use in anemia that cannot respond to iron therapy. Recently it has been elevated once again to a position of great importance in the treatment of certain kinds of anemia. This extraordinary change in the point of view has been commented on by Whipple and Robscheit-Robbins.[1]

The different kinds of iron preparations that have been employed are as various as the different dosages recommended. In general, the activity of different iron preparations is believed to depend on the physical and chemical state of the iron which they contain[5] and on the gross amount of iron which is given in the daily dose.[6] Organic iron preparations are not as effective as inorganic preparations.[7] Reimann and Fritsch[8] found that ferrous chloride and ferrous sulphate in small doses were much more active in producing hemoglobin regeneration than the corresponding ferric salts.

Much further research is necessary before it is learned what is the best form of inorganic iron preparation to employ clinically. There are numerous simple preparations which are potent in types of cases that are known to respond to iron, if they are given in the proper dosage and for an appropriate length of time. An analysis of the important factors in eighty-four cases of hypochromic anemia due to various causes is the basis of the present report.

METHODS

As a rule, venous blood was taken every other day for complete blood studies. The percentage of reticulocytes was determined daily on capillary blood during the first few weeks of treatment. Subsequently, when the hemoglobin reached a level of over 60 per cent of normal and the patient had left the hospital, the hemoglobin and the number of red blood corpuscles were determined at about monthly intervals.

The hemoglobin was determined by the Sahli hemometer, which had been standardized by determinations of the oxygen capacity of the blood by the Van Slyke apparatus. One hundred per cent hemoglobin was taken as the equivalent of 15.6 Gm. per hundred cubic centimeters of blood, or 21 per cent by volume of oxygen capacity. The blood counts were made with United States Bureau of

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Standards' pipettes and counting chambers. For the determination of the reticulocytes, smears of capillary blood were stained supravitally with brilliant cresyl blue, dried, counterstained with Wright's stain and mounted permanently after the methods of Hawes [9] and Cunningham.[10]

Iron was administered by mouth in the form of iron and ammonium citrate (brown scales) or pills of ferrous carbonate, U. S. P., and in a few instances other preparations of inorganic iron were used. Metallic iron in iron and ammonium citrate is about 17 per cent; that is, in each gram of the salt there is about 170 of metallic iron. In each pill of ferrous carbonate there is approximately 30 mg. of metallic iron. These values were used in estimating the dosage and its effects in individual cases.

CLINICAL MATERIAL

Eighty-four patients with hypochromic anemia, who responded well to iron, are under consideration here (table 1). They have been chosen

TABLE 1.—*Classification of Eighty-Four Cases of Hypochromic Anemia Responding to Iron*

Chief Etiologic Factors	Number of Cases	Percentage
Idiopathic hypochromic anemia with achlorhydria or hypochlorhydria	33	39
Chronic blood loss	35	42
Inadequate diet	10	12
Recent pregnancy and inadequate diet	2	
Carcinoma of stomach and chronic blood loss	2	7
Hodgkin's disease and chronic blood loss	1	
Amebic dysentery and chronic blood loss	1	
Total	84	100

from a large group of patients with different forms of anemia who have been carefully studied in this clinic during the past four years. Selection of the cases for the present study has been based arbitrarily on either at least a 1 per cent rise of hemoglobin per day and satisfactory clinical improvement following iron, or the absence of a type of anemia that cannot respond to iron and that has severe complications which experience has shown might hinder the effect of iron. Many of the cases had multiple etiology, for example, poor diet associated with a chronic loss of blood from peptic ulcer, or idiopathic hypochromic anemia with achlorhydria with chronic menorrhagia. No hypochromic pregnancy anemias which respond to iron are included, although there are a few cases of anemia in women in whom a previously terminated pregnancy had undoubtedly contributed to the anemia. There are also included two cases of cancer of the stomach with achlorhydria, one of Hodgkin's disease and one of amebic dysentery, in all of which there had been a pronounced chronic loss of blood.

9. Hawes, J. B.: A Study of the Reticulated Red Blood Corpuscles by Means of Vital Staining Methods: Its Relation to Polychromatophilia and Stippling, Boston M. & S. J. **161**:493, 1909.

10. Cunningham, T. D.: A Method for Permanent Staining of Reticulated Red Cells, Arch. Int. Med. **26**:405 (Oct.) 1920.

In the selection of suitable cases, it is important to exclude cases of anemia due primarily to severe infections, cancer, nephritis and certain other causes, because such complications hinder the action of iron in hypochromic anemia just as they hinder the effect of potent material in pernicious anemia.[11] Many of the eighty-four patients had such complications to a minor degree, but as a rule not sufficiently to hinder greatly the action of the iron medication.

Table 2 gives an additional list of forty-two cases of anemia of various etiologies in which large amounts of iron had been given and no response or a small response obtained. The majority of the patients had color indexes of 1 or more. Slight responses to iron were obtained

TABLE 2.—*Classification of Forty-Two Cases of Anemia in Which Adequate Trial with Iron Gave no Response or Only a Small Response*

Chief Etiologic Factors	Number of Cases
Cancer (stomach, large bowel, pancreas, metastatic)	6
Sepsis (pyelitis, cystitis, pneumonia, tuberculosis, abscess)	9
Chronic nephritis	4
Megalocytic anemia of unknown origin (including aplastic anemia)	8
Myelogenous luekemia	5
Scurvy	4
Hemolytic jaundice	3
Myxedema	1
Cirrhosis of the liver	2
Total number of cases	42

in only a few of the forty-two cases, and the color indexes were below 1. The patients with megalocytic anemia of unknown origin, leukemia, scurvy and hemolytic jaundice had color indexes above 1 and gave absolutely no response to iron. In addition to these cases, eight normal persons showed no response while taking between 0.3 and 2 Gm. of iron daily in the form of ferrous carbonate or iron and ammonium citrate. Mention should also be made of a group of patients with pernicious anemia in whom, during the response of the blood to liver extract or some other potent material, hypochromic anemia developed; they then responded to iron.

FACTORS NECESSARY IN ACCURATE CLINICAL INVESTIGATION OF THE EFFECTS OF IRON THERAPY

Early experiments of Whipple on the acute loss of blood in dogs seemed to lead to the conclusion that iron was of no value in the treat-

11. Minot, G. R., and Castle, W. B.: The Adequate Treatment of Anemia, Ann. Int. Med. **5**:159, 1931.

ment of anemia. The experiments of Williamson and Ets [12] seemed to lead to the same conclusion. Whipple's subsequent experiments showed that iron could be of considerable value in long-standing severe anemia, in which by prolonged severe bleeding and diet control the stores of hemoglobin-building material were reduced until regeneration was much slowed down. This process, which requires so long and is so difficult to produce in the dog, can take place frequently and apparently rather easily in man. This possibility was not recognized formerly, and because iron seemed to fail in so many kinds of anemia, it was thought by many to be useless in all kinds.

Ten years ago, Meulengracht [13] reviewed the difficulties of judging the effectiveness of iron in different kinds of anemia and the necessity of adequate control periods. At that time he was not aware of the value

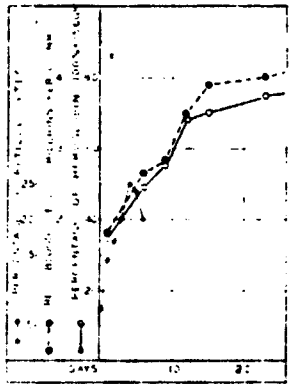

Chart 1.—The blood changes in a man with acute hematemesis from peptic ulcer, apparently having adequate stores of blood-building material. The response of the reticulocytes, red blood cells and hemoglobin to the acute blood loss was good, without the administration of iron, except after the fifteenth day.

of the reticulocyte reaction, but now, since the value of determining the effectiveness of iron by this prompt reaction has been shown, a portion of the difficulties has been overcome. In the clinical investigation of iron therapy in hypochromic anemia there are two factors of primary importance: first, the selection of suitable cases, and second, the establishment of adequate control periods.

Charts 1, 2 and 3, which are illustrative of these facts, represent the blood findings in three men, each of whom entered the hospital with loss of blood from peptic ulcer. Chart 1 represents the blood findings in a

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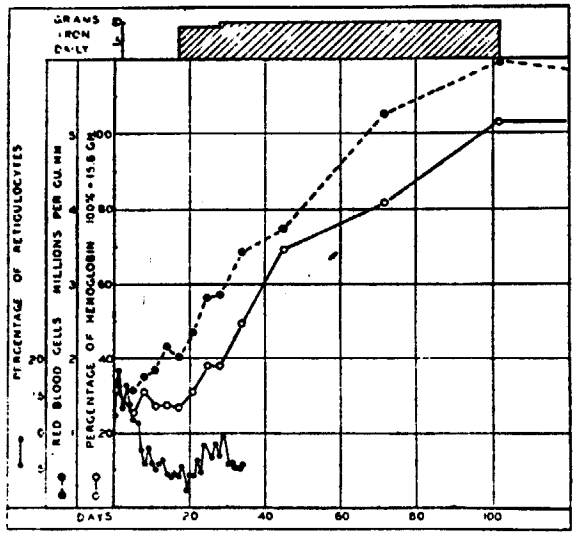

Chart 2.—The blood changes in a man with acute hematemesis from peptic ulcer, apparently having inadequate stores of blood-building material. There was a response of the reticulocytes and red blood cells, but the hemoglobin did not rise until after iron was administered. Note the development of a color index below 1.

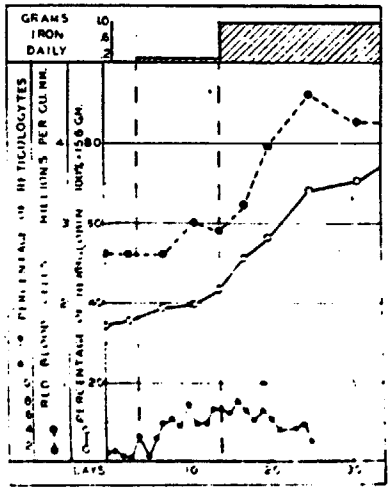

Chart 3 The blood changes in a man with previous chronic blood loss from peptic ulcer. The stores of blood building material had apparently been reduced, and the color index was below 1. A response of reticulocytes, hemoglobin and red blood cells occurred following the administration of 0.1 Gm. of iron. Note the more rapid response of the hemoglobin and red blood cells after the administration of 1 Gm. of metallic iron daily.

man who had acute hematemesis and showed marked anemia when he entered the hospital. The color index was 1; there was a pronounced response of reticulocytes to the loss of blood and a prompt rise of both hemoglobin and red blood cells. He required no iron to produce this response, and iron probably would have caused his blood to rise no faster. He apparently had a good hemoglobin-building reserve to meet the emergency. As recovery took place the color index began to decrease, and it is possible that iron at this later time would have hastened the return of the hemoglobin to normal. Chart 2 represents a similar situation, but an inability to meet the emergency, so that in spite of a high reticulocyte rise due to a loss of blood there were only a slow rise of red blood cells, no rise of hemoglobin and a lowering of the color index. The hemoglobin-building reserve was apparently low, and the administration of iron was of benefit, as shown by the reticulocyte response and the abrupt rise of hemoglobin and red blood cells and the rising color index. Chart 3 shows the blood findings of a man who had a history of a chronic loss of blood from duodenal ulcer. The color index was low, and there was very little evidence of any regenerative ability on the part of the bone marrow. A daily dose of 100 mg. of iron was somewhat effective, but a daily dose of 1 Gm. of iron produced a further reticulocyte response and caused the hemoglobin to rise faster. It must be emphasized that patients were on diets composed almost entirely of milk and cream. These patients were, therefore, from a hematologic point of view, somewhat similar to Whipple's dogs at various stages of bleeding.

The necessity for adequate control periods is well brought out by these cases. It is clear that anemia due to an acute loss of blood is unsuitable for the demonstration of the effectiveness of iron therapy. On the other hand, an acute loss of blood coming after a prolonged period of chronic loss of blood, poor diet or some other condition favoring anemia, such as achlorhydria, may provide an excellent opportunity for testing the blood-building power of iron preparations. In other words, to demonstrate the potency of a preparation of iron in a patient with hypochromic anemia, there must be a sufficient reduction in the patient's reserve of hemoglobin-building material to render him unable to manufacture more than a maintenance amount of hemoglobin.

There are various mechanisms that may produce such a deficiency if one takes into consideration the different types of hypochromic anemia which are known to respond to iron. These may be divided into four main classes: (1) chronic loss of blood; (2) dietary deficiency; (3) gastro intestinal disorders, and (4) pregnancy. It is obvious that the store of hemoglobin-building material may be reduced by a loss of blood. A diet restricted especially in green vegetables, fruit and meat affords an insufficient supply of such material. Gastro-intestinal disorders may interfere with the proper assimilation of this material in the food, as, for example, in the anemia associated with chronic dysentery.[14] This is also well exemplified by the type of anemia known as idiopathic hypochromic anemia with achlorhydria in which the absence of hydrochloric acid in the stomach is associated with an apparent inability to utilize hemoglobin-building substance in the food.[6a] In pregnancy the transfer of hemoglobin-building material from the mother to the fetus explains in part, at least, the frequent production of an iron-responding anemia in the mother, while a change in the secretory ability of the stomach of the mother during pregnancy probably also plays a part.[15] The rôle played by altered gastric function in the production of hypochromic anemia is of such great importance that it must be evaluated in any case even if some other cause for anemia is present.

THE DETERMINATION OF OPTIMAL IRON DOSAGE

Of course, iron dosage has been optimal if the blood response is rapid and if the patient makes satisfactory clinical improvement, but to reach a more definite conception of the appropriate dose certain objective facts are needed. Two kinds of tests have been used in order to approach this conclusion: the first is an arbitrary test; the second, a comparative test. For the arbitrary test the response of the reticulocytes and the rate of hemoglobin rise after iron were compared to certain standards and expressed in terms of percentage of those standards. For the comparative test the response of the reticulocytes and the hemoglobin after a small daily dose of iron for from eight to twelve days was compared to the response after a larger daily dose for a similar period of time. The arbitrary test as employed in the eighty-four cases responding well to iron will be discussed first.

The Arbitrary Test.—The standard for the response of the reticulocytes to iron has been taken from data given by Minot and Heath [16] and is shown in chart 4. Since the height of the reticulocyte rise after iron in hypochromic anemia is inversely proportional to the level of the red blood cells and hemoglobin, considered together, before treatment, the expected height of the reticulocyte rise may be determined for each case by referring to chart 4. This may be done best by averaging the

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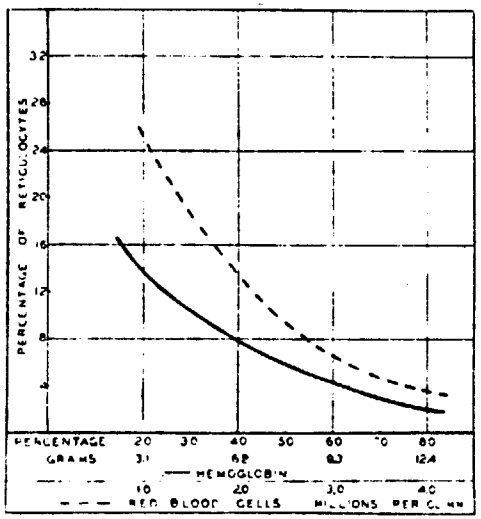

Chart 4.—The average response of the reticulocytes at the peak of their rise in cases of hypochromic anemia responding to iron. The red blood cell and hemoglobin levels, before treatment with iron was started, are recorded as abscissae. Taken from data given by Minot and Heath (Am. J. M. Sc. **183**: 110, 1932).

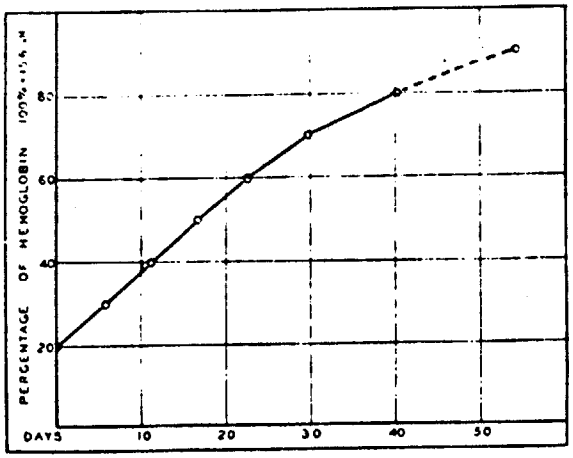

Chart 5.—The average rate of hemoglobin increase in eighty cases of hypochromic anemia during the administration of iron.

percentage of the expected maximal reticulocyte rise at the initial red blood cell level and at the initial hemoglobin level.

The standard for determining the expected rate of hemoglobin rise is shown in chart 5. This figure has been constructed as follows: Based on experience with more than two hundred cases, a rise of 1 per cent (0.16 Gm.) of hemoglobin per day may be assumed to be the lower limit of a satisfactory rate when the initial hemoglobin is below 50 per cent (7.8 Gm.). The number of days required for the hemoglobin to rise each 10 per cent when the rate was 1 per cent a day or faster was determined for each of eighty cases, according to the method described by Josephs.[17] The average was charted and is shown in chart 5. Rises in hemoglobin above 80 per cent occurred only in those patients who continued to improve steadily, whether or not the rate was 1 per cent per day. Therefore, the rate of hemoglobin rise above 80 per cent as expressed in chart 5 is optimal. It was the exception rather than the rule for the hemoglobin in an individual case to continue to rise throughout the entire course of improvement as evenly as the average which is shown in chart 5. In many cases there was considerable irregularity in the rate of hemoglobin increase. The curve for the average, however, compares well with that given by Josephs[17] for anemia in children responding to iron. When the hemoglobin is below 50 per cent, the rate is somewhat slower than that given by Josephs. Above this level it is faster and apparently corresponds more closely to his figures for the hemoglobin rise in children who received copper in addition to iron. This seems to contribute to the evidence that will be mentioned later that copper given in addition to iron in hypochromic anemia in adults does not have any definite influence.

The rate of hemoglobin rise in any given case during the administration of iron may be expressed in percentage of the expected rise as determined from chart 5. Thirty-four per cent of the eighty-four patients had hemoglobin rises due to iron therapy which were satisfactory when judged by this method. Forty-six per cent had satisfactory reticulocyte rises when judged by the arbitrary test for the height of the reticulocyte response to iron. The two tests are therefore fairly comparable and they also afford a standard which requires of a case of hypochromic anemia rapid blood regeneration and therefore adequate iron dosage.

In judging the dosage of iron by this method, the average of the percentages of the expected reticulocyte rise and the expected hemoglobin rise was taken as the criterion. If the average was 100 per cent or over, the dosage arbitrarily was considered optimal; if below 100 per cent, the dosage was considered suboptimal.

17. Josephs, H.: Treatment of Anaemia of Infancy with Iron and Copper, [illegible] Arch. Dis. Childh. **19**:249, 1931.

In only about one half of the cases was there good correlation between the percentage of expected reticulocyte rise and the percentage of expected hemoglobin rise. The discrepancy shown by the remainder of the cases is probably due as much to the errors of the method as it is to the difference in the types of cases and variation in iron dosage. Occasionally in two similar cases there might be, in one, a low reticulocyte response and a high hemoglobin response, and in the other, a low hemoglobin response and a high reticulocyte response. Cases in which the etiology of the anemia was different showed similar discrepancies. The explanation for this state of affairs has not been discovered, but it may be dependent on different reserve powers for the manufacture of cells and for the formation of hemoglobin or on some fundamentally different cellular state of the bone marrow.

After determining in this manner what doses of iron had been optimal, it was apparent at once that the optimal dose varied a great

TABLE 3. *Analysis of the Response to Iron in Thirty-Eight Cases of Hypochromic Anemia in which Patients Were Given 1 Gm. of Metallic Iron as Iron and Ammonium Citrate Daily*

	Total Number of Cases	Number of Cases Having Complications	Number of Cases in Which Reticulocyte Response Was Absent From Hypochromic Anemia to the Normal After Institution of Treatment
Response over 100 per cent of the standard	16	8	2
Response less than 100 per cent of the standard	22	8	11

deal from patient to patient. Thirty four patients, as determined by the methods indicated, received an optimal dosage of iron, averaging 0.72 Gm. daily. Fifty patients received a suboptimal dosage, averaging 0.60 Gm. daily. However, there was a general trend for the smaller doses to produce submaximal responses. For example, in 40 per cent of twenty one cases in which a dose of from 0.35 to 1 Gm. of metallic iron had been given daily, this dose was optimal; on the other hand, in only 19 per cent of twenty six cases in which the dose was from 0.1 to 0.35 Gm. daily was this quantity optimal.

When a dose of about 1 Gm. of iron had been given and the response was submaximal in an individual case, there was often some complication, such as moderate sepsis with fever, that explained the unsatisfactory response. Many of the patients had idiopathic hypochromic anemia, a condition in which the absence of free hydrochloric acid in the stomach may interfere with the proper assimilation of iron. This is well exemplified in the analysis of the responses of thirty-eight patients to whom iron and ammonium citrate, containing 1 Gm. of metallic iron, was given daily (table 3). Of these patients, sixteen, or 42 per cent, had responses over 100 per cent of the standard, which would indicate

that the dosage had been optimal, and of these sixteen patients, five had idiopathic hypochromic anemia, and two additional patients had complications. Twenty-two of the thirty-eight patients, or 58 per cent, had responses less than 100 per cent of the standard, indicating a suboptimal dosage, and of these, eleven patients had idiopathic hypochromic anemia, and eight additional patients had complications. The same results appear when groups of patients receiving a smaller dosage of iron and ammonium citrate and those receiving ferrous carbonate are analyzed in this manner.

In comparing the cases of nine patients who received pills of ferrous carbonate with those of sixty-nine patients who received iron and ammonium citrate, the data showed that a smaller average dose of iron in the form of ferrous carbonate gave greater responses than the larger average dose of iron in the form of iron and ammonium citrate. It is not believed that there is as much discrepancy in the effect of these two iron preparations as this statement would seem to indicate. Evidence will be given subsequently regarding this point.

A considerable difference could be demonstrated when the average response of the thirty-three patients with idiopathic hypochromic anemia was compared to that of the forty-one patients with a chronic loss of blood. The average daily dose of iron was about the same in the two groups of cases, namely, 0.7 Gm. of metallic iron. The average percentage response of the arbitrary standard was maximal in only 29 per cent of the thirty-three cases of idiopathic hypochromic anemia, whereas it was maximal in 53 per cent of the forty-one cases of chronic blood loss.

A detectable response of reticulocytes and hemoglobin was generally noted with small doses of iron (less than 0.1 Gm. of metallic iron daily). One patient, who had chronic blood loss from the uterus, was remarkable in that her blood responded maximally to only 85 mg. of metallic iron in the form of iron and ammonium citrate daily. On the other hand, the following dosages were demonstrated in three patients to be absolutely ineffective: 85, 50 and 60 mg. of metallic iron daily.

It would appear, therefore, that a dose of iron that is optimal for one case of hypochromic anemia may be suboptimal for another, but, in general, small doses of iron are likely to be suboptimal. When a large dose of iron (1 Gm. of metallic iron daily) is given and the response is submaximal there may be some complication. An optimal dose of iron in hypochromic anemia due to an uncomplicated chronic blood loss and without achlorhydria is probably a suboptimal dose in idiopathic hypochromic anemia with achlorhydria. These points are brought out perhaps more strongly in the study of individual cases, and will be further illustrated by the comparative test of iron dosage.

The Comparative Test.—The comparative test is that which has been employed by various investigators in the comparison of the effect of several substances on the formation of blood. The test consists in the uniform daily administration of a substance (in this case a certain dose of iron), followed immediately by the uniform daily administration of a second substance (or a larger dose of iron). In this way any additional reticulocyte response and any faster rate of hemoglobin rise occurring during the administration of a larger dose of iron are quite conclusive of a more effective iron dosage. If no reticulocyte rise is obtained and the anemia is sufficient to permit one when a larger dose of iron is given, and if a response occurred when the first dose was given, the first dose of iron is presumably optimal. A similar state of

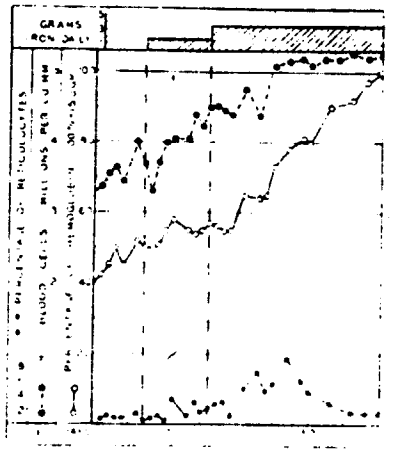

Chart 6.—The blood changes in a case of hypochromic anemia following the daily administration of first 180 mg. of metallic iron as iron and ammonium citrate and subsequently 360 mg. The latter dose was far more effective, as shown by the greater reticulocyte response and the increased rate of hemoglobin formation.

affairs holds for the rate of increase of hemoglobin, but one must judge the rate according to the initial hemoglobin level, for it often is faster at low hemoglobin levels than at high levels. This comparative test is similar to the method used by Reimann and Fritsch* for the demonstration of the superiority of ferrous salts over ferric salts, with the exception that the change between two doses is made abruptly without a period of rest between the doses. The test is illustrated in chart 6, in which 180 mg. of metallic iron, as iron and ammonium citrate daily, produced a slight reticulocyte response and a hemoglobin rise, but 360 mg. was far more effective. The response to the second dose was well over the maximal response, when judged by the arbitrary test, so that in this particular case there is good reason for thinking that 360 mg.

of iron daily was optimal. Table 4 gives the results in all of the cases tested in this manner. It is seen from this table that doses as high as 0.70 Gm. of iron daily were suboptimal, whereas in several other cases smaller doses of iron than this were apparently optimal.

Data from a case of idiopathic hypochromic anemia are shown in chart 7, which illustrates by such a comparative test the fact that the same dose of an iron salt may be more effective if prepared in a particularly suitable form for assimilation. As is well known, pills of ferrous carbonate may be made too firm and with time may become

TABLE 4.—*Comparative Responses of Hypochromic Anemia to Different Doses of Iron (Ferric and Ammonium Citrate Unless Otherwise Indicated)*

Etiologic Factors	Initial Dose, Gm. of Iron	Dose Which Gave Second Reticulocyte Response, Gm. of Iron	Dose Which Gave Faster Hemoglobin Rise, Gm. of Iron	Dose Which Did Not Give Faster Hemoglobin Rise, Gm. of Iron
Idiopathic hypochromic anemia; chronic blood loss	0.085	0.10	0.34	0.10
Restricted diet	0.09	0.18	0.18	....
Chronic blood loss	0.18	0.36	0.36	....
Idiopathic hypochromic anemia	0.19	0.85	0.51	....
Idiopathic hypochromic anemia	0.17	1.00	1.00	....
Chronic and acute blood loss	0.085	1.00	1.00	....
Idiopathic hypochromic anemia; chronic blood loss	0.22	....	1.02	....
Chronic and acute blood loss	0.34	1.02	1.02	....
Idiopathic hypochromic anemia	0.17	1.00	1.00	....
Idiopathic hypochromic anemia	0.21	1.00	....	1.00
Chronic blood loss	0.34	....	....	1.00
Idiopathic hypochromic anemia; chronic arthritis	0.085	....	....	1.00
Idiopathic hypochromic anemia	0.25	....	....	0.34
Chronic blood loss	0.70	....	....	1.00
Restricted diet	0.09*	....	0.35*	....
Chronic blood loss	0.34*	....	0.86	....
Amebic dysentery	0.35*	....	0.52*	....
Restricted diet	0.09*	....	....	0.35*
Postpregnancy, acute blood loss	0.34*	....	....	2.60†

* Ferrous carbonate.
† Ferrum redactum.

hard and resistant. If the preparation is powdered and administered in gelatin capsules, it is in a form more readily available for solution in the gastro-intestinal tract. As is shown in chart 7, the patient received the equivalent of 4 pills of ferrous carbonate (120 mg. of metallic iron) a day for ten days, to which she responded only slightly. When she was given the same number of pills after they had been powdered, a definite second reticulocyte response and a marked rise in the hemoglobin and red blood cell occurred. The equivalent amount of metallic iron given in the form of ferrous sulphate and sodium bicarbonate caused no noticeable increased effect, whereas the equivalent amount of iron in the form of ferrous sulphate and sodium bicarbonate again produced a definite reticulocyte response. These two combinations of substances

with the intention of showing that the reduced form of pills of ferrous carbonate is more effective than the oxidized form (ferric carbonate). The latter form presumably is present to a large extent in pills that have been prepared a long time previous to use. A final moderate reticulocyte response occurred when iron and ammonium citrate in a larger daily dose (1 Gm. of metallic iron) was given. This figure, then, shows how different preparations containing the same dose of iron may vary in their effectiveness and how a larger dose of iron may be more effective than a smaller dose.

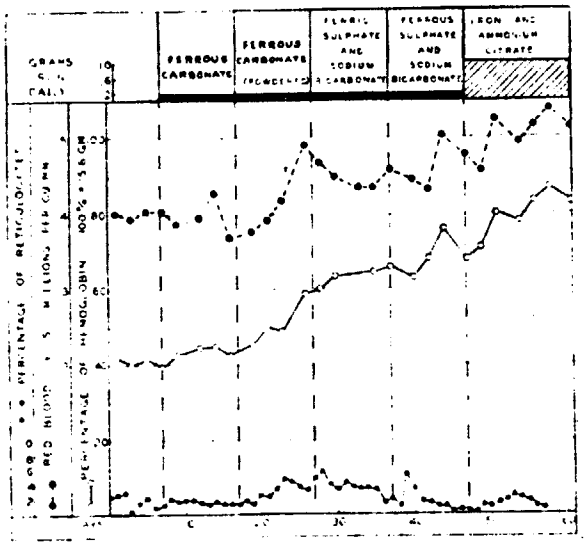

Chart 7. The blood changes in a case of hypochromic anemia during the administration of iron in different physical and chemical states. Note the lack of response to pills of ferrous carbonate given as such, and the excellent response to the same preparation given in powdered form. None of these preparations in a dose of 0.2 Gm. of metallic iron was more effective than 1 Gm. of metallic iron given daily in the form of iron and ammonium citrate.

THE UTILIZATION OF IRON ADMINISTERED ORALLY

It is well known that most of the iron administered orally leaves the body in the feces. To what extent the iron is absorbed or is reexcreted into the intestine is not known. However, the amount of iron contained in the newly formed hemoglobin may serve as an index of the iron retained by the body. This idea was adopted to arrive at a definite conclusion regarding the amount of iron that may have been absorbed and utilized in the building of hemoglobin in the cases studied. The percentage of utilization of iron was determined for each case. For this it is necessary to know the blood volume, which, for the present purpose,

was assumed to be 5 liters for each subject. The grams of hemoglobin per hundred cubic centimeters of blood gained during the period of hemoglobin rise is therefore multiplied by 0.003, a convenient, average figure for the amount of iron in hemoglobin.[18] The final product represents the total amount of iron gained in the circulating blood during the period of hemoglobin rise. The percentage of iron utilization is then determined by dividing the total amount of iron gained in the circulating hemoglobin (× 100) by the amount of iron given orally.

Chart 8 records the percentage of utilization of iron in eighty-one cases. The percentage of utilization of small doses was, of course, much higher than that of large doses. Seven patients who received a total of less than 5 Gm. of metallic iron during the entire period of treatment

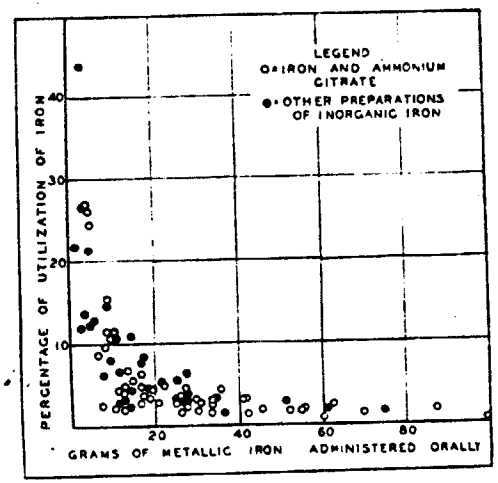

Chart 8.—The percentage of utilization of iron in the formation of new circulating hemoglobin in eighty-one cases of hypochromic anemia during the entire period of hemoglobin rise. The percentage of utilization of iron from iron and ammonium citrate is shown to be similar to that of iron from other preparations.

attained a utilization of over 20 per cent. The few cases in which there were low dosage and low utilization were, for the most part, cases that were complicated by factors known to inhibit the formation of blood, such as sepsis or a malignant process. The figure shows, also, that the percentage of utilization of iron from iron and ammonium citrate was similar to that from the other forms of iron given.

Additional data have shown that the percentage of utilization of iron early in the course of recovery from anemia tends to be considerably higher than the figures recorded in chart 8, which are for the total period

18. Marple, W. P.; Lynch, R., and Howard, J. M.: The Value of Determinations of the Iron Content of Whole Blood, Arch. Int. Med. **47**:883 (June) 1931.

of recovery. Three cases showed, early in the period of recovery, a percentage of utilization of about 50. That iron may be utilized to such a large extent is a fact that is not ordinarily appreciated. The average utilization in all cases during the entire period of recovery was 3.4 per cent. In contrast to this, the utilization in fifty-eight uncomplicated cases, during the first thirty days after iron therapy was commenced, was 11.8 per cent. The average utilization of iron in the thirty-three cases of idiopathic hypochromic anemia during the total period of recovery was 3.1 per cent, and a further contrast is shown by the fact that in the forty-one cases of anemia without achlorhydria due to a chronic blood loss the utilization was 5.5 per cent. It is to be expected that the percentage of utilization of iron in idiopathic hypochromic anemia should be less than that in anemia due to a chronic blood loss without achlorhydria, since the absence of free hydrochloric acid in the gastric contents probably is a factor in preventing adequate absorption of iron.

The information obtained by the determination of the percentage of utilization of iron must not confuse the fact already illustrated by the arbitrary and comparative tests, that larger doses of iron are definitely more valuable than smaller doses. That large doses of iron are utilized to a lesser degree than small doses simply means that the amount of hemoglobin rise, though large, is not proportionately as great as the size of the dose of iron.

A comparison of the parenteral administration of iron with the oral administration of iron as regards the utilization in the building of hemoglobin is interesting, and is discussed in a separate communication.[19] Factors of intestinal absorption do not enter into the problem of the utilization of iron administered parenterally. In cases of hypochromic anemia in which the patients are given iron by the parenteral route, the percentage of utilization approaches 100, and therefore the amount of iron injected is closely related to the amount of iron gained in the circulating hemoglobin.

THE MAINTENANCE DOSE OF IRON IN IDIOPATHIC HYPOCHROMIC ANEMIA

Patients with idiopathic hypochromic anemia, in contrast to those with hypochromic anemia due to a chronic blood loss or other causes, usually require either the continuous administration of iron or frequent courses of iron therapy in order to maintain a normal level of hemoglobin.[20] There have been nineteen patients with this disease who have

19. Heath, C. W.; Strauss, M. B., and Castle, W. B.: Quantitative Aspects of Iron Deficiency in Hypochromic Anemia: The Parenteral Administration of Iron, J. Clin. Investigation **11**:1293 (Nov.) 1932.

20. Heath, C. W.: Idiopathic Hypochromic Anemia with Achlorhydria, M. Clin. North America **15**:1015, 1932. Witts.[2]

en followed for over one year and who omitted iron following the itial recovery of their blood to normal. In all but five patients a efinite drop in the hemoglobin occurred, which soon returned to normal hen adequate iron medication was reinstituted. Of the five patients ho maintained their hemoglobin level after iron was omitted, two howed a return of the acidity of the gastric contents to normal. One f these was a man who for years previous to treatment with iron had dulged excessively in alcohol but did not do so after treatment was mmenced. In these two cases, an improvement in gastric function ndoubtedly permitted a more normal absorption of hemoglobin-building bstances of the food, and therefore rendered the renewal of iron medi-

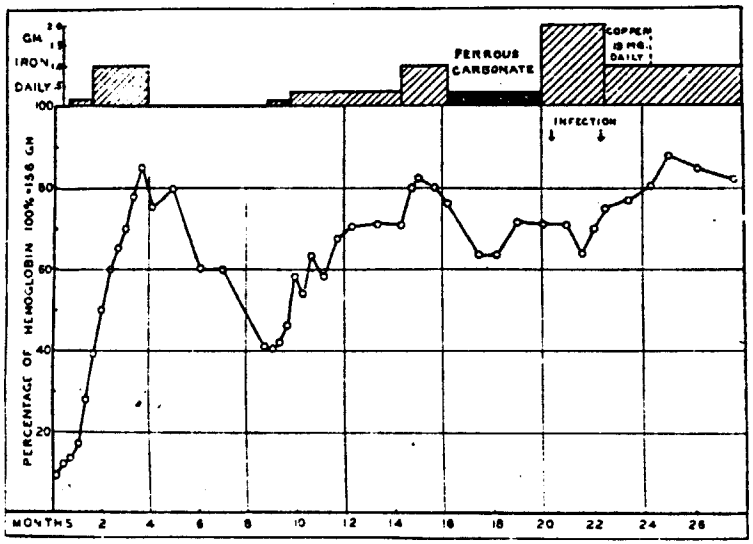

Chart 9.—The changes in the hemoglobin percentage in a case of idiopathic ochromic anemia observed over two years. Iron was administered in the form iron and ammonium citrate except as indicated. Note (1) the prompt decrease hemoglobin when iron was omitted, (2) the rise of hemoglobin over 80 per cent y when 1 Gm. of iron daily in the form of iron and ammonium citrate was n and (3) the failure of 2 Gm. of iron daily to increase the hemoglobin in the of infection.

tion unnecessary. The remaining three patients, who to date have not quired a renewal of iron medication, probably will eventually need it, the complete achlorhydria persists.

Chart 9 represents a prolonged observation on a patient with idiothic hypochromic anemia who has required iron to maintain the moglobin level. The hemoglobin fell gradually over the course of our months when iron was omitted. In addition, the chart demon-

strates a number of interesting points regarding iron therapy. When iron therapy was renewed in this patient, 80 mg. of iron as iron and ammonium citrate daily produced an unsatisfactory rise of hemoglobin (only 10 per cent in one month). Three hundred and seventy-five milligrams apparently produced a somewhat faster response of hemoglobin, but not as rapid as when the patient was first treated and received iron in the large dose of 1 Gm. a day. The hemoglobin fell again when 350 mg. of iron was given in the form of ferrous carbonate. Then the occurrence of an upper respiratory infection with fever lasting for nearly two months apparently prevented the hemoglobin from rising over about 70 per cent, in spite of a daily dose of 2 Gm. of iron. Thirteen milligrams of metallic copper was then given in addition to iron, but it cannot be said that it accomplished more than if iron had been given alone. The hemoglobin rose from 80 to 90 per cent when iron was given without copper.

It is difficult to say what would be the correct minimum maintenance dose of iron for this patient. A daily dose of 375 mg. of iron maintained the hemoglobin at 70 per cent, and it is likely from experience with other cases that if this dosage had been given from the time of the first recovery period it would have kept the patient in a good state of health. This is similar to the maintenance dose suggested by Witts,[2] which he states is not more than one third of the minimum effective curative dose, or about from 0.3 to 0.5 Gm. of iron in the form of ferric and ammonium citrate, or from 0.07 to 0.1 Gm. in the form of ferrous carbonate. It is logical that a smaller dose of iron is needed for the replacement of the normal loss of iron by excretion than for the building up of large amounts of new hemoglobin and the body reserves. However, in one patient 0.12 Gm. of iron daily in the form of ferrous carbonate was insufficient to maintain the hemoglobin at a level of 80 per cent. Iron as iron and ammonium citrate in a daily dose of 0.5 Gm. was insufficient in another patient while she was suffering from a recurrent loss of blood, but when the loss of blood had ceased, a considerably smaller dose was apparently satisfactory.

There is no question but that patients with mild cases of idiopathic hypochromic anemia with achlorhydria will sustain their blood level while taking an adequate diet, but when an additional cause for anemia occurs, such as a chronic or an acute loss of blood, a considerable degree of anemia may be produced which would not occur in a normal person. This can be attributed to small reserves of hemoglobin-building substances in the patient with idiopathic hypochromic anemia, even at a time when the hemoglobin level is nearly normal. On the other hand, certain patients with idiopathic hypochromic anemia, even while taking a proper diet and one rich in iron-containing foods and protein and not suffering from a chronic blood loss or other causes of anemia, cannot maintain their hemoglobin level for more than several weeks without taking relatively large doses of iron daily (from 0.3 to 1 Gm. of iron as iron and ammonium citrate).

The maintenance dose of iron thus seems to vary considerably in different patients and also in individual patients at different times. Much depends on the reserve of iron and possibly other hemoglobin-building substances in the body, on the extent of the gastro-intestinal defect and on the composition of the diet. It is therefore necessary in treating these patients to adjust the maintenance dose of iron to the needs of the given patient, frequent hemoglobin determinations being necessary. It is probable, if large doses of iron are persisted in for a long time after the hemoglobin reaches normal, that the iron stores in the body may be increased. This would allow the hemoglobin level to be maintained for a certain time without iron being administered by mouth. One or two courses of iron therapy a year, lasting several months each, designed to maintain the store of iron in the body, will then be sufficient in many cases to keep the hemoglobin at a normal level, but it seems wiser to administer the drug with regularity.

TOXIC SYMPTOMS FOLLOWING THE ORAL ADMINISTRATION OF IRON AND AMMONIUM CITRATE

Certain disagreeable symptoms are not infrequently met with when iron is administered orally to patients. They have been observed especially when iron and ammonium citrate is given, and the present discussion is concerned with symptoms observed following the administration of this drug, which has been employed to a large extent in this study. Low abdominal cramps and diarrhea are the most common complaints, but these are apt to occur only during the first few days of iron therapy. If the iron medication is persisted in, the diarrhea and cramps usually disappear. Ambulatory patients are much more apt to experience these symptoms than patients who are in bed. Constipation may sometimes accompany the giving of large doses. General malaise, nausea and vomiting, symptoms that follow the parenteral administration of iron and ammonium citrate,[19] have been observed in a few instances. However, these symptoms have never appeared extremely severe or dangerous.

The extremely toxic properties of iron become evident practically only when it is administered parenterally in doses above 16 mg. Some of the toxic symptoms resulting from iron administered orally are due perhaps to an unusually rapid absorption of iron. Hurst[21] has described an unusual case of acute iron poisoning in a patient who was given a large amount of iron orally. It seems wise, therefore, as a

21. Hurst, F. H.: A Case of Iron Encephalopathy, Guy's Hosp. Rep. **81**:243, 1931.

routine, to commence treatment with a small daily dose of iron (2 Gm. of iron and ammonium citrate), gradually increasing it in the course of a few days to the desired amount. Minor symptoms are to be disregarded, since these generally disappear in the course of time as the patient persists in the treatment.

The maximum amount of iron administered orally that is eventually tolerated by patients is, as a rule, very large. One gram of metallic iron, in the form of 6 Gm. of iron and ammonium citrate daily, was taken with ease by the majority of patients. Only three of the sixty-three patients with hypochromic anemia who received iron and ammonium citrate in this dosage had untoward symptoms. In one patient the dose of 0.5 Gm. of metallic iron could not be exceeded because of diarrhea and abdominal cramps. On the other hand, in one patient symptoms of intolerance did not develop until 2 Gm. of metallic iron, as iron and ammonium citrate, was given daily, and even this large dose was well tolerated by several other patients.

THE VALUE OF COPPER THERAPY IN HYPOCHROMIC ANEMIA

Because of the effectiveness of copper in addition to iron on nutritional anemia in rats [22] and on nutritional anemia in children,[17] there has recently been considerable speculation regarding the possible value of copper in hypochromic anemia in adults. The experimental data at hand suggests that the addition of copper adds no beneficial effect to that of iron in adult hypochromic anemia. As described, a curve representing the average maximal hemoglobin response of eighty patients with hypochromic anemia who were treated with large doses of iron has been constructed. Above the level of 50 per cent of hemoglobin, the rate of hemoglobin increase, as shown by this curve, is faster than that shown by a similar curve constructed by Josephs [17] for anemia in children treated by iron alone. This faster rate of hemoglobin rise seems to correspond more closely to Josephs' figures for the rate of hemoglobin increase in children who received copper in addition to iron. Chart 9, as has been explained, illustrates in a case of idiopathic hypochromic anemia the failure of the addition of copper to iron in bringing about a more complete restoration of the hemoglobin to normal than iron alone. Several similar experiments have likewise shown no conclusive evidence that copper added to iron is of value. This is not in accord with the work of Mills [23] and of Adamson and Smith,[24] who

22. Hart, E. B.; Steenbock, H.; Waddell, J., and Elvehjem, C. A.: Iron in Nutrition: VII. Copper as a Supplement to Iron for Hemoglobin Building in the Rat, J. Biol. Chem. **77**:797, 1928. Myers, V. C., and Beard, H. H.: Studies in the Nutritional Anemia of the Rat, J. Biol. Chem. **94**:89, 1931.

23. Mills, E. S.: Idiopathic Hypochromemia, Am. J. M. Sc. **182**:554, 1931.

24. Adamson, J. D., and Smith, F. H.: Chronic Chlorosis, Canad. M. A. J. **24**:793, 1931.

believe that iron and copper are more effective in the treatment of idiopathic hypochromic anemia than iron alone.

It is well known that small amounts of copper and other metals are present as impurities in pharmacopeial preparations of iron salts, and it has been thought that these impurities may have a share in the effectiveness of these preparations. However, a solution of iron and ammonium citrate, freed from the metals of the copper-arsenic group by hydrogen sulphide, produced an excellent response in one case of idiopathic hypochromic anemia.

It is felt that the influence that the addition of copper to iron may have in the treatment of hypochromic anemia in adults is at the most a minor one. Copper may perhaps hasten the recovery of patients with certain types of anemia. The same final results may be attained, however, by large doses of inorganic iron salts. This fact, together with the possible eventual toxic effect of copper, renders it inadvisable to give copper salts as a routine measure in cases of hypochromic anemia.

COMMENT

Evidence has been given showing that the optimal dose of iron administered orally to patients with hypochromic anemia varies considerably in different persons. Therefore, a single optimal dose for all patients cannot be defined. Frequent determinations of the hemoglobin are necessary in each individual case in order to determine whether or not the dose that is being given is adequate. The rule that the hemoglobin should rise at a faster rate than 1 per cent per day when the hemoglobin is below 70 per cent of normal may serve as a rough guide for judging the adequacy of a given quantity of iron.

If the hemoglobin response is much less than 1 per cent per day, doubt is cast on the adequacy of the iron dose, providing the anemia is of a kind that can be expected to respond well to iron, and providing no complications, such as sepsis or severe damage to organs, are present to inhibit the effectiveness of iron.

Sepsis, malignant processes, chronic nephritis, cirrhosis of the liver or other complications, which may themselves be etiologic in anemia, do not contraindicate the use of iron therapy. Hypochromic anemia due to a poor diet or a chronic loss of blood and responding to iron may accompany these conditions. For example, the anorexia accompanying typhoid fever or pulmonary tuberculosis may lead to the consumption of a diet low in many factors, including hemoglobin-building substances; the chronic loss of blood in cancer of the stomach or the hematemesis in cirrhosis of the liver may be the primary cause of hypochromic anemia in conditions responding to iron. The etiologic factors of the anemia in these conditions are usually difficult to judge, but may often be ascertained by an adequate trial with iron and by a complete study of the case.

To be certain of giving an adequate amount of iron in hypochromic anemia, it is necessary to give large doses, such as 6 Gm. of iron and ammonium citrate, corresponding to 1 Gm. of metallic iron daily. Economically, there is no objection to the administration of large amounts of inorganic iron, such as there is to the administration of large amounts of liver extract in the treatment of pernicious anemia, and as a rule large amounts of iron are well tolerated when given orally.

The problem of the oral administration of iron is one involving the quantitative correlation of the dosage with the influence on hematopoiesis. The clinical field for testing iron preparations may be quite well standardized by the careful selection of cases and the employment of adequate control periods. The effectiveness of iron preparations of unknown or doubtful value may then be compared to well known potent preparations. In this way accurate knowledge of the adequacy of treatment of human anemia with iron can be attained. Such knowledge will contribute to the better understanding and control of the etiologic factors in hypochromic anemia.

SUMMARY AND CONCLUSIONS

1. Eighty-four cases of hypochromic anemia have been analyzed with respect to the hematopoietic response to the oral administration of iron.

2. The factors necessary in the accurate clinical investigation of the effects of iron therapy are: (1) the careful selection of suitable cases with regard to their type and etiology and the absence of complications and (2) the establishment of adequate control periods.

3. An arbitrary test has been described whereby the hematopoietic response to iron may be judged quantitatively, and the adequacy of the dosage of iron determined.

4. A comparative test has been described whereby several preparations of iron may be compared with one another as to potency.

5. Optimal dosage of iron in hypochromic anemia, as judged by these tests, varies in different persons. Submaximal responses to large doses of iron are most often present in cases of idiopathic hypochromic anemia with achlorhydria and in cases complicated by sepsis, a malignant process or other conditions. Small doses of iron are, in general, less effective than large doses.

6. The percentage of utilization of orally administered iron, as determined by the total amount of iron gained in the circulating hemoglobin, varies inversely with the size of the dosage. It is possible, during the period of rapid gain of hemoglobin, when iron dosage orally is low, to have as much as 50 per cent of utilization. The average percentage of utilization of iron in the eighty-four cases during the entire period of recovery was 3.4. The percentage utilization of iron in idiopathic hypochromic anemia is less than in uncomplicated hypochromic anemia due to a chronic loss of blood.

7. Patients with idiopathic hypochromic anemia usually require a continuation of iron therapy indefinitely. The maintenance dose of iron in these cases is usually smaller than the dose required for maximum blood regeneration in the period of recovery, but varies with individual cases.

8. Toxic symptoms following the oral administration of iron and ammonium citrate not infrequently occur, but the maximum amount of iron administered orally in this form that is eventually tolerated by the patients is, as a rule, large (from 1 to 2 Gm. daily).

9. It is felt that the influence that the addition of copper to iron may have in the treatment of hypochromic anemia in adults is at the most a minor one, and that it is inadvisable to give copper salts as a routine measure in hypochromic anemia in adults.

10. To be certain of giving adequate amounts of iron in hypochromic anemia, it is necessary to give large doses, such as 6 Gm. of iron and ammonium citrate daily, corresponding to 1 Gm. of metallic iron. Ferrous salts can be equally effective in somewhat smaller doses.

tamic oxalacetic transaminase, 83 units; serum glutamic pyruvic transaminase, 16 units; serum calcium, 8.6 mg/100 cc; and serum phosphorus 3.0 mg/100 cc. A urine culture showed no growth.

The child remained lethargic for several hours in an oxygen tent with persistent red watery diarrhea and the passage of red blood that clotted on the diaper. She developed a fever to 39.2 C (102.4 F) without localizing signs of infection; penicillin and streptomycin were administered with defervescence within 36 hours. Attempts at feeding small quantities of milk at hourly intervals were abandoned because of repeated vomiting. The initial intravenous infusion of dextrose in saline was followed by successive bottles of dextrose in water and subsequently Ringer's lactate solution to alleviate the metabolic acidosis. The child's hemoglobin concentration fell from 13.4 to 11.7 gm/100 cc with a corresponding fall in hematocrit. There was an associated increase in pulse rate to 204 beats per minute, return of dusky color, and unresponsiveness; whole blood (20 ml/kg) was given with marked improvement. X-ray examination of the abdomen for additional radiopaque iron tablets showed dilatation of the small bowel with liquid radiopaque material in several loops of intestine; the liver and spleen were not enlarged.

Additional desferrioxamine hydrochloride (800 mg) was given intravenously 12 and 24 hours after the initial dose. Serial measurements of serum iron, iron binding capacity, urinary iron, and urinary desferrioxamine were obtained (Tables 1 and 2). An electrocardiogram was interpreted as showing sinus tachycardia and depression of S-T segments in leads V_1 through V_6. Twenty hours after admission (24 hours after iron ingestion), the child had the first of five convulsions with cyanosis, generalized twitching, and pooling of secretions. This seizure was controlled with paraldehyde given intramuscularly and amobarbital sodium given intravenously. Further seizures 2, 5, 6, and 12 hours later were controlled each time with intravenously administered amobarbital sodium plus additional maintenance phenobarbital. Examinations during this period showed her pupils to react to light; no abnormalities of the fundi or lungs were noted. An electroencephalogram was characterized by predominant 1-4 second slow high voltage activity mixed with some fast activity; no definite interpretation was made.

Enlargement of the liver 6 cm below the costal margin was first observed though no jaundice was seen. Repetitive blood samples showed the serum electrolyte values to have returned to approximately normal levels with correction of the acidosis. After the blood transfusion the hemoglobin concentration slowly fell to a stable level between 9.5 and 10.2 gm/100 cc with a fall of white blood cell counts to a 12,000 to 19,000 range. By the third day of hospitalization the child showed marked and sustained improvement. She was removed from the oxygen tent. Clear liquid feedings were begun and rapidly changed to a normal diet. Her indwelling catheter was removed and she played happily with no residual abnormalities except for a slight left hemiparesis first noted after her series of convulsions. Mild diarrhea without evidence of pathogenic organisms on three stool cultures responded to neomycin and a combination of kaolin and pectin (Kaopectate)® treatment. The child was returned to her home ten days after admission without any residua of her iron intoxication except the slight hemiparesis. Within a month after discharge all evidence of the hemiparesis had disappeared and a radiographic examination of the upper gastrointestinal tract was entirely normal. Her blood counts showed a hemoglobin concentration of 11.3 gm/100 cc; hematocrit, 36%; white blood cell count, 11,700 with a normal differential.

Table 1.—Measurements of Serum Iron and Iron Binding Capacity During Treatment

Date	Hour of Sample	Serum Iron, μg/100 cc	UIBC* μg/100 cc	TIBC* μg/100 cc	Comments
2/ 9/63	−1	2,550	0	2,550	Pretreatment sample
	2	2,275	0	2,275	800 mg desferrioxamine given IV at hour 0
	5	139	0	139	
2/10/63	11	600	36	636	800 mg desferrioxamine given IV at hours 12 and 24
	24	183	125	308	
	34	115	284	399	
2/11/63	44	113	270	383	
2/12/63	68	120	198	318	

*UIBC and TIBC refer to unsaturated and total iron binding capacity, respectively. UIBC was measured by the Ventura method[19]; serum iron was determined by a modified digestion technique.

Table 2.—Urinary Excretion of Iron and Desferrioxamine During Treatment

Date	Period of Sample	Urine Volume, ml	Urine pH	Urinary Iron Conc, mg/100 cc	Urinary Iron Total, mg	Urinary Desferrioxamine Conc, mg/100 cc
	Initial sample*	19.5	5.2	0.71	0.01	
2/ 9/63	20 min to 2 hr	14	4.6	8.27	1.16	150
	2 to 5 hr	38	5.3	9.25	3.42	6.0
	5 to 6 hr	90	5.8	8.92	8.02	0
	6 to 11 hr	25	6.0	3.59	0.89	8.0
2/10/63	11 to 19 hr	105	6.2	3.39	3.56	48
	19 to 21 hr	43	5.8	4.10	1.77	23
	21 to 35 hr	134	5.6	4.37	5.87	42
2/11/63	35 to 43 hr	113	5.9	0.47	0.53	45.5
	43 to 46 hr	30	5.6	0.42	0.14	32
	Totals	611.5	....	...	25.37	....

*The initial sample was obtained by catheterization 20 minutes after the first dose of desferrioxamine had been given. Additional desferrioxamine was given at 12 and 24 hours. Urinary iron was determined by a modified digestion technique, desferrioxamine was measured colorimetrically.[20]

Comment

This child's story is in most respects typical of that found in acute iron toxicity. Characteristically, the child is a toddler, 12 to 30 months of age, who finds a box or bottle of iron tablets carelessly left within reach by his mother for whom they have been prescribed. The lure of colored tablets that look like candy leads the child to eat a variable number before his activities are halted by his parents or the onset of vomiting. Usually the amount of iron ingested is not precisely known, though fatal doses of ferrous sulfate have varied from 3 to 18 gm, and survival has been reported after doses as high as 15 gm.[1-3]

The effects of ingesting toxic doses of iron have been divided into four phases chronologically.[1] The first phase begins with abdominal pain and vomiting within 30 to 60 minutes after the iron tablets are eaten. Partially dissolved tablets may be vomited along with brown or bloody stomach contents. Soon irritability, pallor, and drowsiness appear along with frequent black or bloody diarrhea. Symptoms of acidosis and cardiovascular collapse may become prominent; coma and death ensue within four to six hours in about 20% of children taking large doses of iron. The second phase consists of a period of improvement in response to treatment of the initial symptoms. Vomiting and diarrhea abate, the symptoms of acidosis and shock improve, and the child appears much less ill. This period, lasting 8 to 16 hours, may

herald the onset of progressive improvement. Often, however, the false security engendered by the transient improvement is rudely shattered by a third phase of progressive cardiovascular collapse, convulsions, coma, and high mortality at about 24 hours after iron ingestion. If this phase can be avoided or treated successfully, the child usually improves rapidly with few difficulties until one or two months later when the fourth phase of gastrointestinal obstruction from scarring occurs; corrective surgery may be required.[5]

Unusual in this child's case is the occurrence of severe iron poisoning due to ferrous gluconate. To our knowledge this is the first reported instance of ferrous gluconate poisoning in a child, though acute iron intoxication after ingestion of this iron compound has been recognized.[6] With only rare exceptions in recent reports, ferrous sulfate alone or in combination with other substances has been responsible for childhood iron poisoning.[1] On the basis of oral toxicity studies in experimental animals, ferrous gluconate is less toxic than ferrous sulfate at comparable doses of iron,[7-9] though the reasons for this difference are not clear. The toxic symptoms in this child were the same as have been described in ferrous sulfate poisoning and suggest the likelihood of severe toxic reactions from any dissociable iron compound that is absorbed rapidly in amounts sufficient to exceed significantly the maximum iron binding capacity of plasma transferrin. The ultimate pathogenesis of many of the symptoms of iron toxicity remains obscure despite extensive morphologic study and animal experimentation.[3,10-12]

On the basis of theoretical considerations, analogy to other iron chelating agents, and animal studies, the use of desferrioxamine in the treatment of acute oral iron toxicity has been suggested by several investigators.[13-15] This drug is a sideramine of microbial origin with a molecular weight of 561. As the soluble hydrochloride salt, it binds 9.3 mg of trivalent iron per 100 mg of chelate with an avidity comparable to that of the plasma iron binding protein, transferrin. Given by mouth, desferrioxamine is not absorbed to any significant degree; in the gut, especially at an acid pH, the drug binds inorganic iron and greatly reduces its absorption. Given intravenously, desferrioxamine combines with iron to form ferrioxamine which is to a large extent excreted in the urine, though some is metabolized in the body. Most of the reported studies of this chelate are concerned with its use in removing excess body iron in diseases of chronic iron storage. Relatively high levels of urinary iron excretion, ease of administration by the intravenous or intramuscular route, lack of clinically significant excretion of other metals, and freedom from serious toxic side effects make the use of desferrioxamine in the removal of excess body iron of considerable promise.[14-16]

The rationale for use of desferrioxamine in the treatment of acute iron intoxication of children is based on the twofold aim of: (1) binding iron circulating in plasma in excess of transferrin binding capacity to render it nontoxic while hastening its excretion in the urine; and (2) binding iron remaining in the gastrointestinal tract to prevent its absorption. Parenteral administration of the drug is used to effect the first aim; administration orally or by gastric tube is designed to achieve the second goal. As with use of other iron-chelating agents such as EDTA, DTPA, and EDDHA, desferrioxamine is but an adjunct to various supportive measures designed to combat symptoms of iron toxicity.

The effectiveness of desferrioxamine in the treatment of the child described in this communication is difficult to evaluate in terms of survival or effects on clinical manifestations. Criteria of drug effects that are more easily analyzed are the changes in serum iron levels and the amount of urinary iron excretion. The fall in serum iron concentration from 2,550μg/100 cc to 139μg/100 cc within five hours after intravenously administered desferrioxamine is much more rapid than has been reported in patients receiving EDTA treatment or in patients receiving no chelating agents.[2] Further evidence of efficient removal of excess iron is the reappearance of small amounts of unsaturated transferrin at 11 hours, despite high circulating serum iron levels (due in part to circulating ferrioxamine). Subsequent serum iron and unsaturated iron binding capacity values remained within the normal range. Likewise, excretion of 25.4 mg of iron in the urine during the first 43 hours of treatment is almost five times the maximum urinary iron excretion reported during a roughly comparable period after repeated EDTA infusions in a patient with an initial serum iron value of 6,260μg/100 cc.[17] No estimate could be made of the amount or sites of distribution of iron that was presumably absorbed in excess of that recovered in the urine. Only normal numbers of hemosiderin granules were observed in the reticuloendothelial cells of a bone marrow aspirate.

The value of desferrioxamine given by gastric tube to this child to prevent further iron absorption cannot be measured. It is possible that the large doses of the drug accentuated the diarrhea due to intestinal irritation, as has been reported.[18] However, the diarrhea was initiated by the iron before desferrioxamine was given; more rapid expulsion of iron bound to the desferrioxamine in the gut may have had a net beneficial effect. Theoretically, desferrioxamine should be of more value than EDTA when given by the oral route, since absorption of iron initially bound to EDTA has been shown to occur,[18] while we have preliminary evidence to suggest that radioiron bound to desferrioxamine given by mouth is not absorbed to any significant extent. Oral administration of desferri-

oxamine soon after ingestion of toxic amounts of iron would seem desirable to minimize iron absorption.

The repeated doses of desferrioxamine (92 mg/kg) given intravenously to this child are entirely empirical. Smaller concentrations of chelating agent might have been effective, though during the initial 12 hours after the first dose only small amounts of free desferrioxamine were excreted in the urine. Successively increasing amounts of iron-free drug appeared after later doses. No harmful side effects were recognized with this dosage schedule, and since data are not available on which to determine the excessive amounts of iron in the body and from this figure to calculate the required dose of chelate, it would seem justifiable to give an excess of the chelating substance.

In combating iron toxicity in children the most important measure is its prevention. This can be accomplished by warning mothers to whom iron tablets are given to keep them out of reach of young children, dispensing iron in bottles with "childproof" closures, and labeling containers with a suitable warning. When these measures fail and a child swallows a toxic dose of iron, a rational plan of treatment for acute oral iron intoxication based on a synthesis of our experience and that of others can be outlined as follows:

1. Rid the stomach of its contents. Induce emesis, lavage the stomach with a large-bore tube to remove undissolved iron tablets. Instill 5 gm of desferrioxamine in aqueous solution or, if this is not immediately available, use a 1% solution of sodium biscarbonate to bind residual iron in a poorly absorbable form. Follow the gastric lavage with an enema to remove iron from the lower bowel. If possible, obtain radiographic confirmation of the success of measures used to remove radiopaque iron from the gastrointestinal tract.

2. Institute measures to combat peripheral vascular collapse. Early intravenous replacement of body fluids and electrolytes using isotonic saline, Ringer's-lactate, plasma, dextran, or whole blood may be needed to treat the hemoconcentration and shock. Injection of an isotonic solution of desferrioxamine hydrochloride (1 gm in 25 ml of water) intravenously is warranted to bind iron circulating in excess of the transferrin binding capacity and hasten its excretion. Calcium-disodium-EDTA (80 mg/kg/24 hours) or calcium-DTPA (20 mg/kg repeated in 4 hours) may be substituted for the desferrioxamine. Repeated doses of chelating agents for 24 to 48 hours are often necessary.

3. Additional measures, often necessary, are treatment of metabolic acidosis with appropriate solutions of sodium bicarbonate. Oxygen treatment and vasopressor agents may help in combating shock. Amobarbital (Amytal), phenobarbital, paraldehyde, or diphenylhydantoin (Dilantin) may be required to control convulsions. Prophylactic antibiotics seem of value when vomiting and aspiration are severe in semicomatose patients.

600 S Kingshighway, St. Louis 10 (Dr. Brown).

The desferrioxamine used in this study was supplied as Desferal by Ciba Pharmaceutical Company, Division of Ciba Corporation, Summit, NJ.

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Studies in Iron Absorption V. Effect of Gastrointestinal Factors on Iron Absorption

By S. HÖGLUND AND P. REIZENSTEIN

PREVIOUS STUDIES showed that general systemic factors like the hemoglobin concentration, serum iron concentration, iron binding capacity, and the plasma iron clearance rate were not correlated to the intestinal iron absorption. Neither did parenteral iron treatment normalize high iron absorption.[1]

The purpose of the present study is to examine such local intestinal factors as the quantity and quality of the iron and of the food present in the intestinal lumen, and also the mucosal iron. Previous studies of, e.g., the absorption of iron from various foodstuffs have been reviewed.[2,3]

MATERIALS

The effect of the iron dose and of ascorbic acid, food, and iron therapy on radioiron absorption was studied. A total of 240 absorption studies were performed in 150 persons.

All studies were performed in healthy male and female volunteers. Normal iron absorption values were established in 24 male and 33 female volunteers.[4] The studies of the effect of food were performed in 33 volunteers, 29 male and 4 female.

Four qualities of iron labelled with ^{59}Fe were used: Ferrous sulphate, (Abbott, specific activity 10.3 mCi/mg. iron, concentration 3.2 μg. iron/ml.) ferrous fumarate, and two qualities of metallic reduced iron prepared by Amersham and Studsvik, Sweden, respectively. In the "coarse" type of reduced iron 48 per cent of the particles were over 30 μ and 23 per cent between 20 μ and 30 μ, while the "fine" type of reduced iron had 0.1 per cent of the particles over 10 μ and 97 per cent about 5 μ. The iron enrichment of the flour was 40 mg./Kg., and the total iron content thus became in flour 50 mg./Kg. and in bread 35 mg./Kg.

For the studies of the effect of iron dose and of ascorbic acid, ferrous fumarate was used because convenient radioactive tablets could be obtained (Ferrosan Drug Co., Malmö). The tablets were labelled with 2–3 μCi ^{59}Fe. One kind contained only 10 mg. iron, the other also 200 mg. ascorbic acid. It has been demonstrated previously that no significant absorption difference exists between ferrous sulphate and ferrous fumarate.[5]

To study the effect of sifted flour on the absorption of iron used to enrich flour, 30 Gm. slices of bread baked with sifted wheat flour were used. Each slice contained about 1 mg. iron and 1–1.5 μCi ^{59}Fe.

The absorption of the reduced iron customarily used to enrich flour and of ferrous sulphate was studied. The baking was performed thanks to Dr. T. Widhe, Swedish Cooperative Society.

To study the difference between the effect of a carbohydrate-rich and a fat-rich meal upon iron absorption, two standard meals were prepared and called "porridge" and "porridge and cream," respectively, where the "porridge" was prepared from nonsifted

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From the Department of Internal Medicine (Section of Hematology and Gastroenterology and Blood Bank, Karolinska Hospital and King Gustaf V Research Institute, Stockholm, Sweden.

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Table 1.—Composition of the Meals Studied[7]

Meal Classification	Fat Gm.	Carbohydrate Gm.	Protein Gm.	Iron mg.	Iron Absorption Index§ Men	Women
Sifted flour (60 Gm.)	1.9	31.1	5.1	1.1	1.041	—
Coarse ground flour *	2.6	30.4	8.7	1.6	0.226	0.164
Coarse ground flour † and cream	37.5	28.5	7.5	1.5	0.279	0.124
Complete meal ‡	25.5	44.3	16.0	2.7	0.084	—

* 36 Gm. hulled oats and 100 Gm. skimmed milk. The hulled oats contain approximately 6.0 μg. phytic acid phosphorous per Gm.[11-14]

† 36 Gm. hulled oats and 100 Gm. whipping cream.

‡ Consisting of approximately 2 slices of bread (30 Gm.) and butter (10 Gm.), ham (20 Gm.) and lettuce (10 Gm.), tomato (10 Gm.), cheese (20 Gm.), 1 cup of coffee (100 Gm.) and 2 sweet rolls (50 Gm.).

§ Mean for men and women compared to normal absorption. The index is lower in women who have a higher normal absorption.

coarse-ground flour. To study the difference between coarse-ground and sifted flour, hulled oats (porridge) and wheat bread were compared. In addition, a "complete meal" was studied, data about which were taken from a previous publication.[6] The composition of the different meals is shown in Table 1.

The radioiron was added to the porridge in the form of ferrous sulphate. The effect of a complete meal on iron absorption was studied by giving the patients a ferrous sulphate solution immediately after eating.

The studies of the effect of the luminal iron concentration and of ascorbic acid were performed in 25 healthy female volunteers. In all these volunteers, a careful history was taken to exclude blood donors and patients receiving medical attention.

To study the effect of oral iron treatment, i.e., of a possible intracellular iron concentration increase in the intestinal mucosa, subjects with iron deficiency but otherwise healthy were desired. Twenty-six male blood donor volunteers were selected. They are described elsewhere.[1]

Methods

The methods used for determination of serum iron concentration, total iron binding capacity, plasma iron clearance rate and iron absorption have been described,[4,8] as have the statistical methods.[9] Iron absorption was measured using radioactive iron and a whole body counter. The radioactive background of fasting subjects was registered and 0.25 mg. $^{59}Fe^{++}$ administered in a drink of water. One hour after administration the 100 per cent radioactivity value was measured, and 2 weeks later the body retention of the test dose was registered.

Studies of the effect of ascorbic acid, fat and oral iron treatment on absorption were planned as crossover studies, i.e., each person served as his own control. It was not possible to perform all studies in a single group of subjects for practical reasons and because the number of permissible tracer studies in healthy controls is limited. For this reason, and because iron absorption in, e.g., men is not directly comparable to that in women, an "iron absorption index" is used. It is the ratio between the mean absorption found in the group given a particular nutrient or form of iron, and the normal mean absorption of ferrous iron in the relevant comparison group.

Results and Discussion

Lumenal Iron Concentration

Previous studies of the relation between the iron absorption and the dose of iron used have been reviewed.[2] Table 2 shows the present results, and

Table 2.—Effect of Luminal Iron Concentration

Oral Iron Dose	No. of Subjects	Mean Age, Years	Mean Serum Iron Conc. mg./100 ml.	Iron Absorption, Per Cent of Dose	
				Mean	S.E. of Mean
0.25 mg. iron as ferrous sulfate	33	26	0.116	43.5	4.4
10 mg. iron as ferrous fumarate	25	22	0.108	17.6	2.7

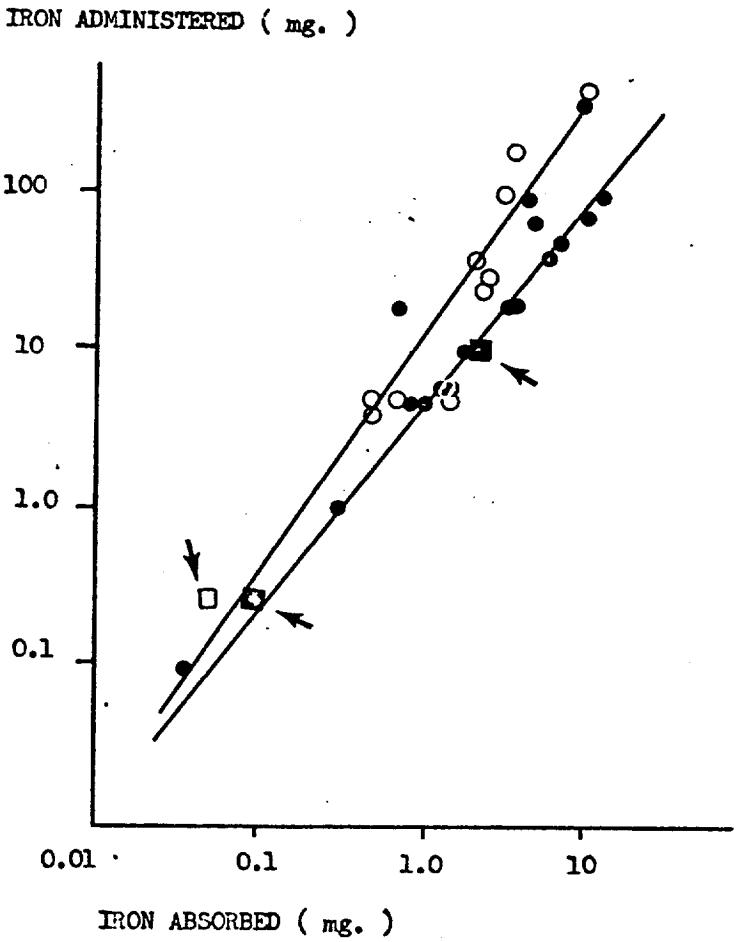

Fig. 1.—Absorption of iron from different iron doses. Data from literature (circles)[15] and present investigations (squares). The oblique lines are regression lines for men (open circles) and women (closed circles) respectively. The present data, open square (men) and closed squares (women), have been superimposed.

Figure 1 shows the good agreement between present and previous data. It is also seen that the lumenal iron concentration does influence absorption—the percentage absorption decreases with increasing concentration. The difference is statistically significant ($P < 0.01$). However, a fortyfold increase in concentration decreases absorption by little more than one half.

Table 3.—Effect of Quantity of Bread Eaten on Iron Absorption

Form of Iron	Amt. of Bread (Gm.)	Iron Content mg.	No. of Subjects (male)	Mean Absorption	S.E. of Mean

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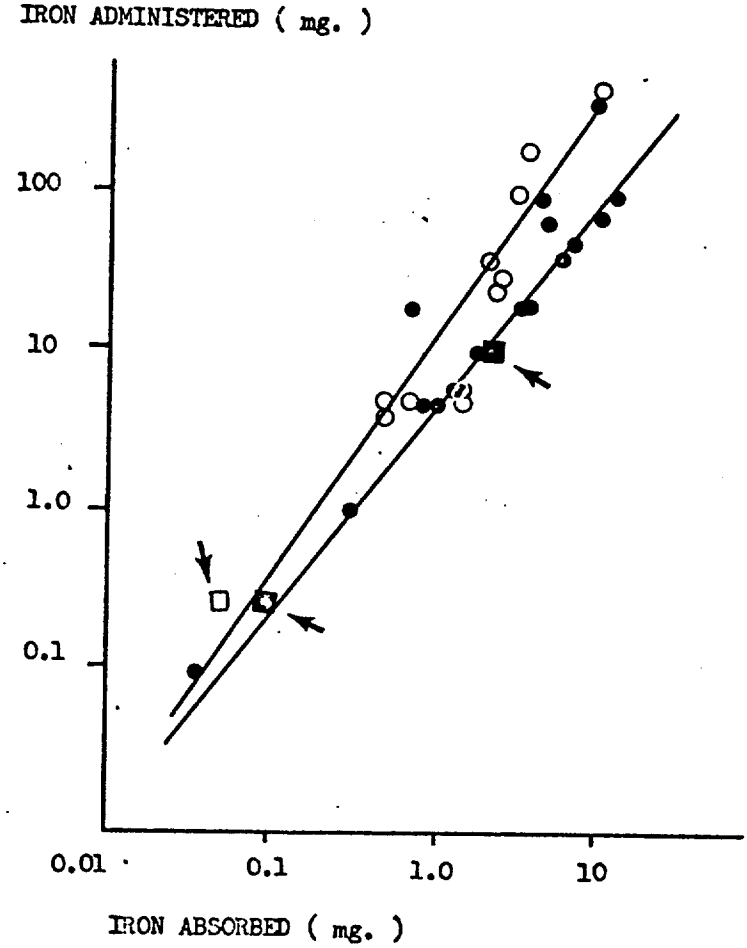

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Table 3.—Effect of Quantity of Bread Eaten on Iron Absorption

Form of Iron	Amt. of Bread (Gm.)	Iron Content mg.	No. of Subjects (male)	Mean Absorption	S.E. of Mean
^{59}Fe-metallic*	25	0.8	5	12.1	5.9
"	90	3	8	7.0	2.9
^{59}Fe SO_4	40	1.3	4	26.8	8.6
"	90	3	4	12.6	5.9

* Radioiron given as fine grain reduced iron used to enrich flour.

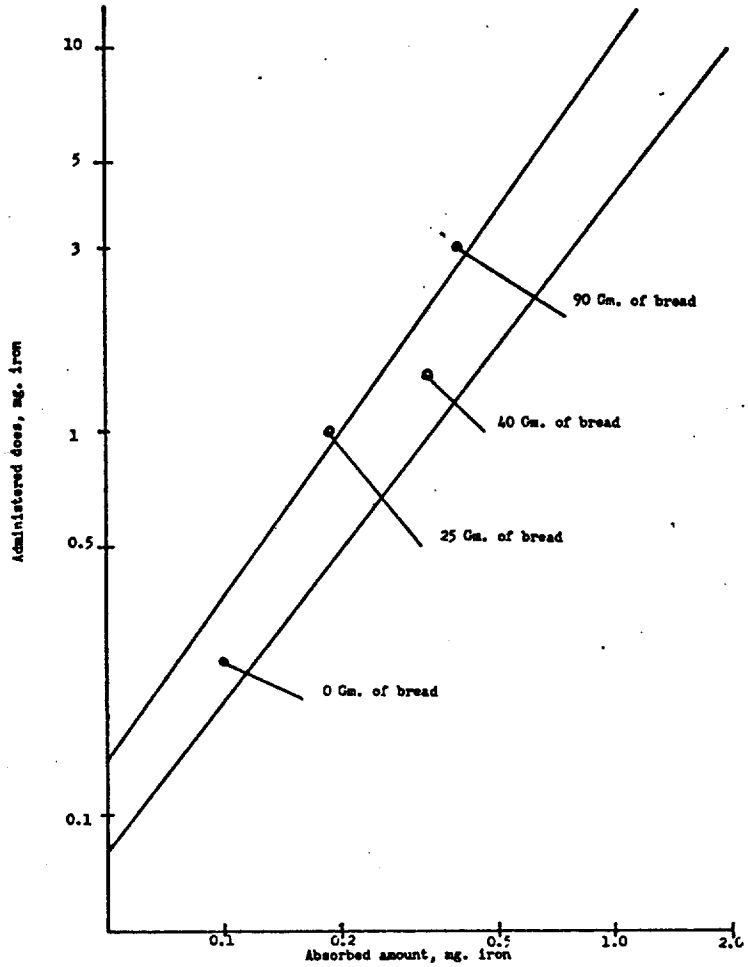

Fig. 2.—Absorption response to dose of iron salt (lines) and bread iron (circles). The oblique lines[15] represent normal absorption of iron salt in men (left) and women (right). The figure shows that the absorption response is as good for bread iron as for iron salt, in spite of an increasing bulk of bread.

Table 3 shows the effect of food quantity upon iron absorption. Numerically, a decrease in absorption is seen for both metallic and soluble iron when the quantity is increased, but statistically it is not significant. Not only the food quantity, but also the increased iron content of the intestine, from

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FROM THE DEPARTMENT OF INTERNAL MEDICINE II (HEAD: E. WASSÉN, M.D.), SAHLGRENSKA SJUKHUSET, UNIVERSITY OF GÖTEBORG, GÖTEBORG, SWEDEN, AND THE BLOOD BANK (HEAD: L. RYTTINGER, M.D.), SAHLGRENSKA SJUKHUSET, GÖTEBORG, SWEDEN.

SIDE-EFFECTS OF ORAL IRON THERAPY

A double-blind study of different iron compounds in tablet form

By

LEIF HALLBERG, LARS RYTTINGER AND LENNART SÖLVELL

The use of oral iron in the treatment of patients with iron deficiency is usually effective and is seldom associated with any serious practical difficulties. In some patients, however, certain difficulties may arise. a) In patients with continuous heavy losses of iron, e.g. with heavy menstrual blood losses, the effectiveness of the treatment is counteracted which results in a slower hemoglobin response than normally and a difficulty to reach the individually optimal hemoglobin value. b) Some patient experience side-effects of oral iron therapy which may necessitate a reduction of the dose or discontinuation of the therapy. From a practical point of view, the aim of iron therapy is to obtain a maximal absorption with a minimum of side-effects. To evaluate the effectiveness of oral iron preparations, it is thus necessary to know both their absorbability and their side-effects.

Numerous reports have been published on the side-effects of various iron compounds. However, only a few authors have used an adequate experimental technic e.g. the double-blind technic and the use of a placebo (3). In two studies where the double-blind and placebo technic was used, the iron dose was as low as 35 mg three times daily (4, 5). No difference in the frequency of side-effects was observed between iron and placebo tablets. The side-effects were thus considered psychological in origin at the dosage given. In two other studies with higher iron doses and with an adequate technique, no significant difference in the frequency of side-effects was observed between placebo and ferrous iron tablets (1, 2). It is well-known, however, that some patients actually do not tolerate oral iron at the dosage usually prescribed. The commonly recommended dosage is in the range 150—300 mg elemental iron daily. Therefore, in the present study, the dose range was chosen so as to correspond with a commonly used iron dosage.

The present study comprises three series. In the first series, the side-effects of placebo and ferrous sulphate tablets were compared. In the other two series, other iron compounds were included. In the latter series placebo and ferrous sulphate tablets were used as references.

The studies were made in 1496 subjects who had served as blood donors for a long time at the Blood Bank of Sahlgren's Hospital in Göteborg and who had not received previous regular iron supplementation. The present studies were included in a program of designing an adequate iron prophylaxis to regular blood donors.

After a blood donation, the subjects received tablets in a bottle labelled "Iron tablets for blood donors". A code number was also printed on the label. The bottle contained tablets for 14 days of medication. The subjects were instructed to take the tablets 3 times daily and were informed of the importance of iron medication after blood donations. They did not know that later on they would be questioned about the medication.

The bottles containing the different iron tablets were randomly distributed to the blood donors. The code was not broken until the study was completed.

Two weeks after the blood donation, a questionnaire was sent to the subjects together with a letter explaining the importance of getting an early and complete reply.

In the questionnaire, the subjects were asked if they had taken the tablets as instructed. They were also asked if they had noticed any marked change in their bowel habits (constipation, diarrhea) during the treatment period. Furthermore they were asked to state whether they had had any symptoms of nausea, vomiting, heartburn, epigastric discomfort or other symptoms which they ascribed to the therapy. The subjects were requested to state if any of the side-effects had been of such a severity as to interfere with the continuation of the therapy.

In each series, the tablets were of the same size and colour and had the same pharmaceutical properties and the same coating. All iron tablets in each series contained the same amount of elemental ferrous iron.

RESULTS

I. *Comparison between placebo and ferrous sulphate tablets.*

In this series (Series 1), 393 subjects were included — 195 received placebo tablets and 198 ferrous sulphate tablets. Two tablets were taken three times daily. Each iron tablet contained 37 mg elemental iron. The daily iron dose was thus 222 mg

Replies were obtained from 344 subjects. Side-effects were reported by 13.6 per cent of the subjects receiving placebo tablets and 4.1 per cent discontinued the therapy due to side-effects. The corresponding figures for the subjects receiving iron tablets were 22.9 and 8.0 per cent. The difference in frequency of side-effects was statistically significant

frequency of subjects discontinuing the therapy was not statistically significant ($X^2=2.21$, $P>0.1$). The type and frequency of side-effects encountered in the two groups are given in Table I (Series 1). Minor side-effects comprised various symptoms, e.g. slight abdominal distension, constipation, or slight degree of loose stools. Higher frequencies of diarrhea, nausea, and minor side-effects were observed in the ferrous sulphate group than in the placebo group.

In the placebo group the minor side-effects were slight constipation — in the iron group, slight abdominal distension, slight constipation, or loose stools.

Table II shows the number of subjects in the groups who discontinued the therapy due to side-effects and the type of side-effects reported. Some subjects reported more than one cause for the discontinuation.

II. *Comparison between placebo tablets and iron tablets containing ferrous sulphate, ferrous fumarate, or ferrous gluconate.*

In this series (Series 2), 477 subjects were included — 119 received placebo tablets, 120 ferrous sulphate, 118 ferrous fumarate, and 120 ferrous gluconate. Two tablets were taken three times daily. Each iron tablet contained 37 mg elemental iron and the total daily dose was thus 222 mg.

Replies were obtained from 447 subjects. Side-effects were reported by 13.9 per cent of the subjects receiving placebo tablets. The corresponding figure for the ferrous sulphate group was 27.9 per cent, for the ferrous fumarate group 26.4 per cent, and for the ferrous gluconate group 31.5 per cent.

Statistical analyses using the X^2-test showed that there were no significant differences in the frequency of side-effects between the iron groups ($P>0.05$). Comparisons between the placebo group and each of the iron groups showed that the differences in the frequency of side-effects were statistically significant ($P<0.05$).

The type and frequency of side-effects encountered in the four groups are given in Table I (Series 2). There were no marked differences between the iron groups. The minor side-effects were of the same type as reported for Series 1.

In the placebo group, 0.9 per cent discontinued the treatment due to side effects. The corresponding figure for the ferrous sulphate group was 9.9 per cent, for the ferrous fumarate group 5.5 per cent, and for the ferrous gluconate group 5.4 per cent. Table II shows the number of subjects in different groups who discontinued the therapy due to side-effects. The type of side-effects reported is also shown in this table.

Statistical analyses were made using the X^2-test. Falsely significant differences can be obtained, however, when there are frequencies below 5. Therefore X^2 was calculated with Yates correction. At the 5 per cent level there was no significant difference in the frequency of subjects discontinuing the iron therapy between the different iron groups. When the iron groups were put together into one group and compared with the placebo group the difference in frequency was significant.

TABLE I. *Side-effects of different iron preparations. Three series.*

Series	Compound	Dosage: Tablets	Dosage: mg Fe	Subjects: ♂	Subjects: ♀	Subjects: Total	Replies: ♂	Replies: ♀	Replies: Total	Replies: Per cent of total subj.	Subjects with side effects: ♂	Subjects with side effects: ♀	Subjects with side effects: Total	Subjects with side effects: Per cent of replies
1.	Placebo	2 × 3	— —	162	33	195	139	30	169	86.7	17	6	23	13.6
	Ferrous sulphate	2 × 3	74 × 3	163	35	198	144	31	175	88.4	34	6	40	22.9
2.	Placebo	2 × 3	— —	108	11	119	105	10	115	96.6	14	2	16	13.9
	Ferrous sulphate	2 × 3	74 × 3	107	13	120	98	13	111	92.5	26	5	31	27.9
	Ferrous fumarate	2 × 3	74 × 3	103	15	118	95	15	110	93.2	25	4	29	26.4
	Ferrous gluconate	2 × 3	74 × 3	98	22	120	89	22	111	92.5	27	8	35	31.5
3.	Placebo	1 × 3	— —	170	30	200	148	29	177	88.5	16	6	22	12.4
	Ferrous sulphate	1 × 3	60 × 3	152	43	195	128	42	170	87.2	30	15	45	26.5
	Ferrous glycine sulphate	1 × 3	60 × 3	172	28	200	155	25	180	90.0	33	11	44	24.4
	Ferrous gluconate	1 × 3	60 × 3	161	35	196	144	34	178	90.8	39	9	48	27.0

III. *Comparison between placebo tablets and iron tablets containing ferrous sulphate, ferrous glycine sulphate, and ferrous gluconate.*

This series (Series 3) comprised 791 subjects — 200 received placebo tablets, 195 ferrous sulphate, 200 ferrous glycine sulphate, and 196 ferrous gluconate. One tablet was taken three times daily. Each iron tablet contained 60 mg elemental iron. The daily iron dose was thus 180 mg.

Replies were obtained from 705 subjects. Side-effects were reported by 12.4 per cent of the subjects receiving placebo tablets. The corresponding figure for the ferrous sulphate group was 26.5 per cent, for the ferrous glycine sulphate group 24.4 per cent, and for the ferrous gluconate group 27.0 per cent. There was no significant difference in the frequency of side-effects between the iron groups.

Comparisons between the placebo group and each of the iron groups showed that the differences in the frequency of side-effects were statistically significant ($P<0.05$).

The type and frequency of side-effects

TYPE OF SIDE EFFECT																						
Constipation				Diarrhea				Heartburn				Nausea				Epigastric pain				Minor side effects			
♂	♀	Total	Per cent of replies	♂	♀	Total	Per cent of replies	♂	♀	Total	Per cent of replies	♂	♀	Total	Per cent of replies	♂	♀	Total	Per cent of replies	♂	♀	Total	Per cent of replies
10	5	15	8.9	1	0	1	0.6	5	1	6	3.6	1	0	1	0.6	3	0	3	1.8	2	0	2	1.2
9	5	14	8.0	9	1	10	5.7	3	1	4	2.3	9	1	10	5.7	3	1	4	2.3	11	0	11	6.3
3	2	5	4.3	1	0	1	0.9	3	0	3	2.6	0	0	0	0	1	0	1	0.9	6	0	6	5.2
10	1	11	9.9	7	0	7	6.3	4	0	4	3.6	4	2	6	5.4	6	2	8	7.2	7	2	9	8.1
7	4	11	10.0	7	0	7	6.4	4	2	6	5.5	0	0	0	0	3	0	3	2.7	9	0	9	8.2
12	3	15	13.5	6	1	7	6.3	3	2	5	4.5	4	0	4	3.6	4	1	5	4.5	5	3	8	7.2
8	3	11	6.2	4	2	6	3.4	3	0	3	1.7	2	1	3	1.7	0	0	0	0	3	0	3	1.7
11	8	19	11.2	7	4	11	6.5	3	0	3	1.8	3	2	5	2.9	2	4	6	3.5	9	2	11	6.5
10	8	18	10.0	8	0	8	4.4	2	1	3	1.7	4	1	5	2.8	7	2	9	5.0	9	2	11	6.1
16	7	23	12.9	11	0	11	6.2	3	1	4	2.2	5	1	6	3.4	4	2	6	3.4	9	1	10	5.6

ncountered in the four groups are given n Table I (Series 3). There were no narked differences between the iron roups. The minor side-effects were of he same type as reported for Series 1 nd 2.

In the placebo group, 1.1 per cent iscontinued the treatment due to side-ffects. The corresponding figure for the errous sulphate group was 8.8 per cent, or the ferrous glycine sulphate group 7.8 per cent, and for the ferrous gluconate group 7.3 per cent. Table II shows the number of subjects in different groups who discontinued the therapy due to side-effects.

The results of the statistical analyses were the same as in Series 3 i.e. there was a statistically significant difference between the placebo group and the iron groups but not between the different iron groups.

TABLE II. *Subjects discontinuing the iron therapy, and type of side-effects in these subjects.*

Series	Compound	Dosage		Subjects discontinuing therapy			
		Tablets	mg Fe	♂	♀	Total	Per cent of replies
1.	Placebo	2 × 3	— —	4	3	7	4.1
	Ferrous sulphate	2 × 3	74 × 3	10	4	14	8.0
2.	Placebo	2 × 3	— —	1	0	1	0.9
	Ferrous sulphate	2 × 3	74 × 3	10	1	11	9.9
	Ferrous fumarate	2 × 3	74 × 3	6	0	6	5.5
	Ferrous gluconate	2 × 3	74 × 3	6	0	6	5.4
3.	Placebo	1 × 3	— —	1	1	2	1.1
	Ferrous sulphate	1 × 3	60 × 3	8	7	15	8.8
	Ferrous glycine sulphate	1 × 3	60 × 3	11	3	14	7.8
	Ferrous gluconate	1 × 3	60 × 3	8	5	13	7.3

DISCUSSION

In numerous reports it has been stated that a certain iron compound has less side-effects than others. However, in these studies no adequate technic has been used. Very often the incidence of side-effects of one compound has been compared with the incidence of other compounds observed in other series or by other investigators.

The significant difference in the frequency of side-effects between subjects taking placebo and subjects taking iron tablets observed in this study clearly shows that there are side-effects which must be ascribed to the iron medication. There was a difference not only in the frequency of subjects who had side-effects but also in the frequency of subjects who discontinued the therapy. Many factors probably affect the incidence of side-effects observed in a study, i.e. the knowledge that iron is given, the

TYPE OF SIDE EFFECT																								
Constipation				Diarrhea				Heartburn				Nausea				Epigastric pain				Minor side effects			
♂	♀	Total	Per cent of replies	♂	♀	Total	Per cent of replies	♂	♀	Total	Per cent of replies	♂	♀	Total	Per cent of replies	♂	♀	Total	Per cent of replies	♂	♀	Total	Per cent of replies
2	3	5	3.0	0	0	0	0	2	0	2	1.2	0	0	0	0	1	0	1	0.6	0	0	0	0
5	3	8	4.6	2	1	3	1.7	0	1	1	0.6	4	1	5	2.9	1	1	2	1.1	1	0	1	0.6
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0.9
5	0	5	4.5	2	0	2	1.8	0	0	0	0	3	1	4	3.6	2	0	2	1.8	2	0	2	1.8
1	0	1	0.9	2	0	2	1.8	1	0	1	0.9	0	0	0	0	1	0	1	0.9	1	0	1	0.9
4	0	4	3.6	1	0	1	0.9	0	0	0	0	1	0	1	0.9	0	0	0	0	0	0	0	0
1	1	2	1.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	3	5	2.9	2	4	6	3.5	0	0	0	0	1	0	1	0.6	1	3	4	2.4	3	0	3	1.8
5	3	8	4.4	2	0	2	1.1	1	0	1	0.6	2	1	3	1.7	3	1	4	2.2	0	0	0	0
3	5	8	4.5	3	0	3	1.7	0	0	0	0	1	1	2	1.1	1	0	1	0.6	1	0	1	0.6

anticipation that iron tablets may have side-effects, the way the subjects are questioned etc.

The previous inability to observe a difference between sideeffects of placebo and iron tablets can be explained by the low dosage of iron (35 mg elemental iron three times dialy) used in two of the earlier studies (4. 5). It is reasonable to assume that there is a dosage level below which most subjects do not experience any sideeffects of iron tablets. In the two previous studies in which higher doses were used -- 100 mg 3 times daily (2) and 80 mg 4 times daily (1) — no significant difference in frequency of side-effects between the placebo and the iron groups was found. These observations are thus not consistent with the present ones. The reason is probably differences in the experimental design between the studies. The present findings that iron tablets have side-effects and that some subjects did not continue the medication due to side-effects is based on consistent significant observations in three separate series.

The significant differences between placebo and iron tablets thus show that the present method was sufficiently sensitive to study side-effects of oral iron therapy. Moreover, the observed agreement in incidence of side-effects between the three series shows that the accuracy of the method was very good. This is exemplified by the small variation in the incidence of side-effects of the placebo tablets (12.4 to 13.9 per cent) and of the ferrous sulphate tablets (22.9 to 27.9 per cent).

Another conclusion that can be made on the basis of the present study is that different ferrous compounds have the same incidence and the same type of side-effects when the same amount of elemental iron is administered.

These observations make it improbable that there are iron compounds which are tolerated better than ferrous sulphate To make oral iron therapy more effective it is thus necessary to focus the interest on the absorbability of different iron compounds and on factors which may increase the absorption of iron.

SUMMARY

Using a double-blind and placebo technic, the side-effects of tablets containing different iron compounds were compared in 1496 subjects. Three separate series, all of which included placebo and ferrous sulphate tablets were studied. The agreement between the results obtained in different series was very good

Ferrous sulphate, ferrous gluconate ferrous fumarate, and ferrous glycin sulphate had almost the same incidenc and type of side-effects. The incidenc was significantly higher than when placeb tablets were given.

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THE ABSORPTION OF IRON BY NINE COLLEGE WOMEN FROM FERRIC ORTHOPHOSPHATE AND FERROUS SULPHATE INCORPORATED INTO BREAD

(Publication No. 15,598)

Inez Kemble Harrill, Ph.D.
Cornell University, 1955

Young women were maintained on a diet of low iron content for a control period of 28 days. Following this ferrous sulphate or ferric orthophosphate were incorporated into bread and fed in addition for 28-day periods. The iron content of the food and the feces was determined. The mean intake of iron during the control period, ferrous sulphate and ferric phosphate enrichment periods was 5.43 mg., 12.75 mg., and 12.40 mg., respectively. The amount of iron excreted in the feces averaged 4.41 mg. during the control period and 11.51 mg. and 11.28 mg. during the ferrous sulphate period and the ferric phosphate period, respectively.

The amount of iron absorbed from each iron preparation was determined by finding the difference between the increase in the amount of iron in the food and the increase in the amount of iron in the feces due to the addition of fortified bread to the diet. The mean for the amount of iron absorbed from ferrous sulphate bread was 0.26 mg. or four per cent and the mean for the amount of iron absorbed from the ferric orthophosphate bread was 0.19 mg. or three per cent. On each iron preparation four subjects apparently absorbed no iron from the added preparations and two absorbed large amounts. The small amount of iron absorbed from the ferrous sulphate and ferric phosphate incorporated into bread indicates that the addition of a larger amount of the iron preparations for the fortification of bread and flour would be of nutritional value.

The mean hemoglobin level for 9 subjects was 13.5 gm. per 100 ml. of blood. There was no significant change of the hemoglobin values during the experiment. The mean serum iron level for six subjects determined eight months following the termination of the study was 142.8 mcg. with a range of 97.0 mcg. through 193.0 mcg. per 100 ml. of serum. The subject who had the highest hemoglobin concentration and serum iron level absorbed an unusually large amount of iron. Apparently the high percentage of absorption was not due to poor stores.

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Table 4.—Effect of Ascorbic Acid *

Oral Dose	No. of Subjects	Iron Absorption	
		Mean	S.E. of Mean
10 mg. ferrous fumarate	9	9.8	1.4
10 mg. ferrous fumarate + 200 mg. ascorbic acid	9	28.3	4.3

* Absorption given as per cent of administered dose. Iron and ascorbic acid were combined in a pharmaceutically stable tablet.

0.8 to 3.0 mg. Fe, must be considered. As may be calculated from Figure 1 and Table 2 the increased iron dose alone would be expected to cause a maximum decrease in absorption of 10 per cent of the administered dose, i.e. from 33 to 23 per cent, (Fig. 1) and it is thus probable that volume increase alone does not significantly decrease iron absorption. This is further illustrated in Figure 2 where the oblique lines indicate the correlation between $FeSO_4$ dose and absorption and where absorption from the different bread iron doses falls within or close to this normal area.

Ascorbic acid facilitates absorption even of soluble ferrous iron. Table 4 shows that with the pharmaceutical preparation used (Ferrocevit, Ferrosan, Malmö, Sweden) a statistically significant threefold increase in absorption is obtained in healthy controls. The effect found is larger than the 50 per cent increase described earlier,[5] probably because of differences in method and material.

The present results do not prove that therapeutic benefit is obtained by giving combinations of iron and ascorbic acid to patients with manifest iron deficiency, who have a high absorption of iron even without ascorbic acid. However, even blood donors[5] with a probable iron deficiency do seem to absorb more iron in combination with ascorbic acid (and succinic acid). This suggests that such a benefit may be obtained. Also, an ascorbic acid supplement in itself, especially to persons with nutritional defects, may be valuable—possibly more so than a succinic acid supplement. Since most people forget to take their tablets unless they are taken with meals, the ascorbic acid may also be useful in overcoming the absorption-inhibition caused by food.

Bread baked from sifted wheat flour did not appear to inhibit the absorption of ferrous iron (Tables 1 and 5). Since about half of the iron in Swedish diets derives from bread,[10] and since iron deficiency is a common disease, this finding may be of relevance.

The mean iron absorption after coarse ground flour porridge was lower than that found after bread baked from sifted flour (Table 5), but these studies were not performed in the same subjects. It is nevertheless quite probable that the difference is due to the hull fraction present in the coarse ground flour.

No further decrease in absorption resulted when fat was added to the porridge in spite of the possibility that iron soaps may be formed in the intestine.

Thus, neither the lumenal iron concentration (Fig. 1), nor the food volume alone (Fig. 2), nor carbohydrates (cereals) or fat (cream) alone (Table 5

Table 5.—Iron Absorption with Food

Main* Food Constituents	Approximate Iron Quantity mg.	No. of Patients	Subject Category	Percentage Absorption of Iron Mean S.E.	Confidence Level Statistical Significance of Inhibition §
Sifted flour	1	8	Men	17.5 ± 5.3	$P > 0.05$
Coarse ground flour (Porridge)	1.6	3	Men	4.3 ± 2.6	
		4	Women	6.5 ± 3.1	$0.001 < P < 0.01$
		7	All	5.6 ± 1.9	
Coarse ground flour + fat (Porridge and cream)	1.5	3	Men	5.3 ± 3.7 †	
		4	Women	4.9 ± 2.0	$0.001 < P < 0.01$
		7	All	5.1 ± 1.8	
Complete meal (Carbohydrates + fat + protein)	2.9	7	Men	1.6 ± 0.2 ‡	$0.001 < P < 0.01$

* Obviously some fat and protein is present in all foods studied.

† The mean difference, in the same subjects, between iron absorption from porridge and porridge and cream was 0.5 ± 3.6 per cent.

‡ The same subjects absorbed 18 ± 1.5 per cent of ferrous sulfate.[6]

§ All mean values compared to 24 normal men (19.0 ± 2.3) and 27 normal women (39.7 ± 4.4).

seem to explain the inhibition of absorption seen after a complete meal.[6] The present studies do not show whether this inhibition is a sum effect of the effects already mentioned, or whether it is secondary to a particular food constituent not yet examined. Neither fat nor sifted flour per se decreased absorption in a statistically significant fashion, but coarse-ground flour seems to, and a complete meal does.

The sifted wheat flour contained about 0.08 mg. phytic acid phosphorous per mg. iron (an equimolar amount would be about 3.2 mg.) and the coarse ground oats contained about twice as much. It is believed that phytase, which is present in the wheat flour but not in the oats, is activated during the baking process and splits the phytic acid molecule, thereby interfering with its possibility to bind iron.

Higher extent of phytic acid and lack of phytase in coarse ground flour compared to sifted flour may partly explain the greater inhibition of iron absorption.

Iron Quality

Table 6 demonstrates that the grain size of reduced iron is important for iron absorption. The coarser the reduced iron, the less is absorbed, but even of the fine quality considerably less is absorbed than of ferrous iron. The difference is statistically significant (Table 7). These findings may be important for three reasons: Two thirds of the bread iron is derived from enrichment; bread is responsible for about 50 per cent of the iron in Swedish food; and iron deficiency is a common condition.[4] It is conceivable that the decreased intake of iron secondary to decreased caloric requirements must be compensated by a qualitatively and quantitatively superior iron enrichment of the food.

Table 6.—Absorption of Iron Used to Enrich Bread *

Form of Iron in Bread	Grain Size	No. of Measurements	Absorption, % M S.E.
Reduced iron	Fine †	13	9.0 ± 2.3
Reduced iron	Coarse ‡	9	3.0 ± 0.8
Fe SO_4	—	8	19.8 ± 5.7

* 1 slice bread (30 Gm.) contains 1 mg. radioiron added to the flour.
† 0.1 per cent > 10 μ, 97 per cent about 5 μ.
‡ 48 per cent > 30 μ, 23 per cent about 25 μ.

Table 7.—Statistical Significance of Some Differences in Crossover Studies

Effect Studied	Difference Between	No. of Subjects	Difference, per cent of dose ± S.E. of mean	Significance
Ascorbic acid	Absorption with and without ascorbic acid	9 female	19.3 ± 4.2	$P < 0.01$
Form of iron in bread	^{59}Fe-absorption after $FeSO_4$ in bread and coarse metallic iron	8 male	17.4 ± 5.3	$0.01 < P < 0.02$
Complete meal	^{59}Fe-absorption in subjects fasting and after meal (6)	6 male	16.1 ± 1.3	$P < 0.01$
"Mucosal iron"	^{59}Fe-absorption before and after oral iron treatment	26 male blood donors	19.7 ± 6.0	$P < 0.01$

"Mucosal Iron Concentration"

The quotation marks indicate that it is only an assumption that the effect of oral iron treatment upon iron absorption can be attributed to "mucosal iron concentration." The assumption is supported by the finding that neither a fortyfold increase of the lumenal iron concentration (Table 2) nor parenteral iron treatment[1] had a comparable effect.

The results of the oral treatment are seen in Table 7. Although the calculated increase in body iron after a month of treatment is only about one fortieth of the normal total body iron, and although a similar increase by parenteral treatment did not affect absorption significantly, iron absorption is halved. The decrease in iron absorption was statistically highly significant (Table 7). The relation between this decrease and concomitant changes in general systemic factors is discussed elsewhere.[1] A further study is in progress to examine the time relation between oral treatment and the normalization of absorption.

Summary

1. Since previous studies could not demonstrate that any of several general plasma factors played a major role in intestinal iron absorption, local intestinal factors were examined in 240 iron absorption studies on 150 healthy subjects.

2. When the iron dose was increased 40 times, from 0.25 to 10 mg. the percentage absorption was halved.

3. Trebling the quantity of food (bread) in the intestine did not significantly decrease absorption.

4. Ascorbic acid in the intestinal lumen trebled the absorption even of ferrous iron. A stable pharmaceutical combination of iron and ascorbic acid was tested.

5. Sifted flour did not seem to inhibit the absorption of ferrous iron, but coarse ground flour did. When fat was added, no further decrease in absorption was found although iron soaps may be formed.

6. A further decrease in absorption was found after a complete meal.

7. When fine grain reduced iron was used to enrich flour—this is done in all Swedish flour—absorption was 50 per cent lower, and when a coarser grain reduced iron was used 85 per cent lower, than when ferrous sulfate was used for enrichment.

8. When oral iron treatment was given to persons with high iron absorption, absorption was decreased to normal.

SUMMARIO IN INTERLINGUA

1. Viste que previe studios non succeedeva a demonstrar que ulle de plure general factores plasmatic ha un rolo major in le absorption intestinal de ferro, local factores intestinal esseva examinate in 240 studios del absorption de ferro in 150 subjectos normal.

2. Quando le dose de ferro esseva augmentate ab 0,25 ad 10 mg, i.e., per un factor de 40, le absorption procentual esseva reducite per un medietate. Le triplication del quantitate de alimento (pan) in le intestino non reduceva le absorption de maniera significative.

4. Acido ascorbic in le lumine intestinal triplicava le absorption mesmo de ferro ferrose. Un stabile combination pharmaceutic de ferro e acido ascorbic esseva testate.

5. Farina cribrate non pareva inhibir le absorption de ferro ferrose, sed farina molite plus grossiermente habeva iste effecto. Quando grassia esseva addite, nulle declino additional in le absorption esseva trovate, ben que le formation de sapon a ferro occurreva.

6. Un declino additional in le absorption esseva trovate post un repasto complete.

7. Quando un reducite ferro a grano fin esseva usate pro inricchir le farina — isto es le costume in Sveda pro omne farina — le absorption esseva plus basse per 50 pro cento, e quando reducite ferro a grano plus grossier esseva usate, illo esseva plus basse per 85 pro cento que quando sulfato ferrose esseva usate in le inricchimento.

8. Quando un tractamento oral a ferro esseva applicate a subjectos con un alte absorption de ferro, le absorption esseva reducite a nivellos normal.

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HARRY GOLD, M.D.

PROFESSOR OF CLINICAL PHARMACOLOGY, CORNELL UNIVERSITY MEDICAL COLLEGE

AND

McKEEN CATTELL, M.D.

PROFESSOR OF PHARMACOLOGY, CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK, NEW YORK

A REVIEW OF THE TOXICITY OF IRON COMPOUNDS

BY JAMES O. HOPPE, PH.D.
MEMBER

G. MARIE AGNEW MARCELLI, M.S.
RESEARCH ASSOCIATE

AND

MAURICE L. TAINTER, M.D.
DIRECTOR

(From the Sterling-Winthrop Research Institute, Rensselaer, New York)

In recent years a number of reports have appeared on the poisoning of young children by ferrous sulfate tablets. Iron salts have been freely used as medicaments for thousands of years so that the physician ordinarily is quite unconcerned about any toxic potentialities, particularly when the compound is to be given orally. Since this lack of fear of toxicity seemed inconsistent with the clinical toxicological reports, it became desirable to review the literature on comparative toxicity of various iron salts in experimental animals and in accidental poisonings in patients. The literature survey also indicated the desirability of a new direct experimental comparison under critical conditions of the tolerance of ferrous gluconate and ferrous sulfate. This study has been carried out and is reported elsewhere[38].

History of Iron Therapy. The origin of iron therapy is obscure in the dimness of prehistoric medical experience. It is known to have been employed by the ancient Hindus, Egyptians and Greeks. Iron (apparently as the sulfate) was one of the few inorganic medicines described in the old Egyptian pharmacopoeias. Similarly, in an attempt to bestow the strength of iron upon a patient, Greek physicians administered the metal as a cure for

weakness (which is one of the prominent symptoms of anemia). Water in which red hot iron had been quenched or in which swords had rusted was frequently the medicinal form of iron[30]. Hippocrates also recommended it for both diarrhea and constipation.

In the seventeenth century Sydenham wrote of the treatment of chlorosis by iron[32]:

"To the worn out or languid blood it gives a spur or fillip whereby the animal spirits which before lay prostrate and sunken under their own weight are raised and excited. Clear proof of this is found in the effect of steel in chlorosis. The pulse gains strength, the face (no longer pale and death-like) a fresh ruddy color."

Iron was first shown to be present in the blood in the eighteenth century and Menghini[50] demonstrated that iron in the blood could be increased by feeding foods rich in that substance. Still another significant advance, in the same century, was William Cullen's prophetic warning that the good effects of iron were often missed because of too small doses.

But iron therapy reached its golden age with Pierre Blaud's introduction of his famous pill in 1831[30]. For most of the remainder of the century eulogies proclaiming iron first among all therapeutic agents were common. Then, Bunge, Quincke, and others, finally convinced physicians that inorganic iron was hardly absorbed at all. This, coupled with the unsatisfactory results of injudicious use of iron in all types of anemia, led at last to the "Dark Ages" of iron therapy[21]. Ferrous sulfate, for instance, was not even considered for internal use in the United States Dispensatory of 1918. It was only after the first quarter of the present century that the value of large doses of iron, where iron was needed, was again recognized[29,70].

The frequency of poisoning by iron appears to be a direct function of the fashion in iron therapy in any given period. Opinions on the noxious effects of this substance have varied widely. Sydenham maintained that "iron may be given in the largest doses without inconvenience." However, in 1851, Orfila[54] pleaded, because of the increasing number of accidental and homicidal poisonings from iron salts, for recognition of the toxicity of ferrous sulfate which he and Smith had demonstrated 36 years earlier in *1815*. The law courts of France[7,8,14,44,79] and Italy[28,59] in the middle of the nineteenth century, confused by the divided opinions on the toxicity of iron salts, turned to medical men who carried out animal experiments. Based on these results, iron salts were ruled "poisons" in the legal sense and their administration for felonious purpose constituted "attempted premeditated murder"[59,78].

However, after the turn of the present century, the dispute as to the absorbability of inorganic iron led to the disappearance of iron preparations from the family medicine chest, and iron poisonings vanished. Then observe the trend in thinking on iron as knowledge of the older clinical and experimental reports dimmed and was lost:

1904: "Sufficient evidence exists that ferrous sulfate and ferric chloride have toxic properties"[55].

1928: "Fatal poisoning in man is exceptional"[69].

1934: "Cases of poisoning due to ingestion of iron are extremely rare"[25].

1941: "General intoxication from orally administered iron therapy is unknown"[30].

With the discovery that orally administered iron is utilized in the body and with the gradual acceptance of its safety, particularly when compared to

reactions after parenteral administration[39,43,60], ferrous iron has returned to prominence. In fact so popular is this remedy that five hospitals serving 400,000 people dispensed half a million iron pills in a recent period of 6 months[21].

Since ferrous sulfate tablets are often brightly colored and sugar- or chocolate-coated, they may have a tempting appeal for small children, and hence lead to accidental poisoning[16,22,76]. When reports of such cases began to appear, interest in the toxicology of iron quickened. However, the older literature seems largely to have escaped attention. Thus, Somers[71] reported that "examination of the literature failed to reveal earlier reports of ill effects from orally administered iron compounds. Further . . . we have been unable to find any account of pharmacological investigation into the action of iron given by mouth."

Attention was drawn to this problem in 1952 by the editors of the *Journal of Pediatrics*[10] as follows:

"It is puzzling to understand why medicinal iron preparations, which have been used for generations and which have been looked upon as almost innocuous in overdosage according to medical texts, should first be reported in the last few years as a cause of severe and fatal accidental poisoning in young children. It is obvious that the potential dangers of medicinal iron as a cause of accidental poisoning should be better known to physicians and the public. . . ."

In view of the conflicting evidence and, more important, the increasing frequency of fatalities following the oral ingestion of iron salts, particularly in infants and children, it has become desirable to take a more extensive look at the literature on the toxicity of the iron preparations available for medicinal use. There are summarized below the results of this search of the literature.

Toxicology of Iron Salts in Animals. Estimates of the median lethal dose for several iron preparations by various routes of administration in experimental animals are summarized in Tables 1 to 4. An attempt has been made to express the data in terms of the median lethal dose as mg./kg., both as the salt and its equivalent in terms of ionic iron. The source of the data is indicated in each instance by the reference.

Particularly striking is the fact that relatively few attempts have been made to establish the acute toxicity of these preparations in experimental animals with any degree of precision. In many instances considerable difficulty was encountered by us in attempting, from the published data, to establish the form of the preparation used, the manner in which it was given, duration of the observations, and number of animals employed. Thus, finding a means of expressing the data in standard terminology was a definite problem. From Tables 1 and 4, it becomes possible to arrange the compounds in order of increasing oral toxicity in animals, as follows:

Compound	*Species*	*Estimated Oral LD mg./kg.*
Ferrous gluconate	mouse	6600
	guinea pig	2100
	rabbit	3500
Ferric ammonium citrate	mouse	5000
	guinea pig	1750
	rabbit	2800
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	mouse	4500
	guinea pig	1500
	rabbit	3000
	cat	>500
	dog	800
Ferric chloride	mouse	1500
	guinea pig	600
	rabbit	1200
Ferrous chloride	rat	600
	rabbit	1000

TABLE 1.—TOXICITY OF FERROUS SULFATE (ORAL ADMINISTRATION)

Species	Dose* As Salt	Dose* As Fe^{++}	LD_{50} mg./kg. As Salt	LD_{50} mg./kg. As Fe^{++}	Comments	Ref.
Mouse	..	..	4500	900	$FeSO_4$ crystalline	71
	..	..	4100	1000	$FeSO_4$ as "Fersolate"	71
	..	..	..	710		13
Frog	..	29.4 mg.	..	..	Fatal. Animal wts. 35 to 45 gm.	73
Rat	..	73.6 mg.	..	..	1/3 died. Animal wts. 100 to 200 gm.	73
Guinea pig	400 mg.	..	..	..	Fatal 18½ hrs. Fe as "Fersolate"	25
	200 mg.	..	..	..	Fatal in ½ hr.	25
	400 mg.	..	..	..	Survived } Fatal dose equals	25
	600 mg.	..	..	..	Fatal } 65 mg./64 gm.	25
	800 mg.	..	..	..	Fatal	25
	..	..	1500	300	$FeSO_4$ crystalline	71
	..	..	1250	300	$FeSO_4$ as "Fersolate"	71
Rabbit	..	..	3000	600	$FeSO_4$ crystalline	71
	..	..	3000	720	$FeSO_4$ "Fersolate"	71
	3000 mg./kg.	..	..	..	With 3 gm. $NaHCO_3$. Survived	71
	3000 mg.	..	..	..	Fatal	79
	..	368 mg./kg.	..	..	Ill but survived	73
	..	736 mg./kg.	..	..	Fatal	73
	1000 mg.	..	..	..	In 30 to 50 gm. corn meal. No ill effects	28
	4327 mg./kg.	..	..	..	Fatal 3 to 4 hours	28
	1869 mg./kg.	..	..	..	Fatal < 1 hour	28
	769 mg./kg.	..	..	..	Fatal 1½ hours	28
	540 mg./kg.	..	..	..	Ill but survived	28
Cat	1000 mg.	240 mg.	..	..	5 "Fersolate" tablets. Survived	25
Dog	2000 mg.	..	..	..	Ill but survived	69
	8000 mg.	..	..	..	Fatal in 26 hrs.	54
	930 mg./kg.	..	..	..	Fed in cornmeal. Ill but survived	28

* Where the dose is given only as the salt, the authors have not indicated the state of hydration and, therefore, the absolute iron content cannot be calculated.

TABLE 2.—TOXICITY OF FERROUS SULFATE (INTRAVENOUS ADMINISTRATION)

Species	Dose* As Salt	Dose* As Fe^{++}	LD_{50} mg./kg. As Salt	LD_{50} mg./kg. As Fe^{++}	Comments	Ref.
Mouse	..	..	..	13.8		13
	..	..	..	11		51
Rabbit	..	30–60 mg./kg.	..	..	Fatal dose lies in this range	47
	..	10–15 mg./kg.	..	..	Fatal in nine hours	69
Cat	..	30–60 mg./kg.	..	..	Fatal dose lies in this range	47
Dog	..	30 mg./kg.	..	..	Lethal dose for dog	47
	400–500 mg.	..	..	..	Ill but survived	54
	..	10 mg./kg.	..	..	No effect	74
	..	20 mg./kg.	..	..	Fatal in three hours	74
	..	70 mg./kg.	..	..	Immediate death	74

* Where the dose is given only as the salt, the authors have not indicated the state of hydration and, therefore, the absolute iron content cannot be calculated.

TABLE 3. TOXICITY OF FERROUS SULFATE (RECTAL AND TOPICAL ADMINISTRATION)

Species	Route of Admin's.	Dose* As Salt	Dose* As Fe^{++}	Tissue Irritation	Comments	Ref.
Rat	Rectal	..	36.8 mg.	..	Fatal in 3 hrs.	73
		..	73.6 mg.	..	Died in ½ hr } Wt. 100 to 200 gm.	73
		..	73.6 mg.	..	Died in 4 hrs.	73
Rabbit	Rectal	..	368 mg./kg.	..	Fatal in 6 hours	73
Dog	Topical	8000 mg.	..	Intense	Fatal in 12 to 27 hours when applied to cellular tissue of thigh	54

* Where the dose is given only as the salt, the authors have not indicated the state of hydration and, therefore, the absolute iron content cannot be calculated.

TABLE 4.—TOXICITY OF OTHER IRON SALTS

Salt	*Species*	*Route of Admin.*	*Dose**		*LD_{50} mg./kg.*		*Comments*	*R*
			As Salt	*As Fe^{++}*	*As Salt*	*As Fe^{++}*		
Ferrous gluconate	Mouse	Oral			6600	1100	Figures are those reported by the author who indicated iron content as 16 2/3%	
	Guinea pig	"			2100	350		
	Rabbit	"			3500	580		
Ferrous chloride	Frog	"		22 mg.			Fatal in 24 hrs. Wt. 35 to 45 gm.	
	"	"		33 mg.			Fatal in 4 hrs.	
	Rat	"		14 mg.			Somewhat ill	
	"	"		18 mg.			Somewhat ill	Wt. 100 to 200 gm.
	"	"		28 mg.			1/2 died after 24 hrs.	
	"	"		56 mg.			Fatal 7/7 in ½ to 30 hrs.	
	Rabbit	"		168 mg./kg.			No effect	
	"	"		224 mg./kg.			2/2 no effect	
	"	"		252 mg./kg.			Fatal in 24 hrs.	
	"	"		280 mg./kg.			Fatal in 5/5 in 24 to 48 hrs. Higher doses all fatal	
Ferrous carb.	Mouse	"			31000	3800	As Bland's Pills	
	Guinea pig	"			16000	2000	" " "	
	Rabbit	"			17800	2220	" " "	
Ferric chloride	Mouse	"			1500	500		
	"	"				840		
	Guinea pig	"			600	200		
	Rabbit	"			1200	400		
	Dog	"	3.75–5 gm.				Fatal in 27 to 30 hrs.	
	"	"	2.5 gm.				Severely ill one week, impaired digestive process	
Ferric ammon. cit.	Mouse	"			3000	1000		
	Guinea pig	"			1750	350		
	Rabbit	"			2800	560		
Sodium ferricit.	Rat	"		37.2 mg./kg.			1/2 dead in 2 hrs.	
	Rabbit	"		186 mg./kg.			Fatal in 4 hrs	
Ferrous chloride	Dog	I.V.		10 mg./kg.			No effect	
	"	I.V.		30 mg./kg.			Fatal in 8 hrs.	
Ferric chloride	Mouse	I.V.				18.5		
Ferric ammon. cit.	Mouse	I.V.				16.5		
Sodium ferricit.	Frog	S.C.		5–10 mg.			Produced paralysis	
	Rabbit	I.V.		25 mg./kg.			Average lethal dose	
	Cat	I.V.		30-60 mg./kg.			Lethal. No symptoms for 3 days	
	Dog	I.V.		20-50 mg./kg.			Lethal	
Ferrous bicarb.	Dog	I.V.		5 mg./kg.			No effect	
	"	I.V.		10 mg./kg.			Fatal in 3 hrs.	
Ferric tartrate	Mouse	I.V.				16.5		
Ferrous chloride	Rat	Rectal		28 mg.			Fatal in 48 hrs.	
	"	"		56 mg.			Fatal in 5 min.	
	Rabbit	"		280 mg./kg.			Fatal in 1–2 in 5 hours	
Sodium ferricit.	Rat	"		37 mg./kg.			Fatal in 5 hrs.	
	Rabbit	"		186 mg./kg.			Fatal in 4 hrs.	

* Where dose is given only as the salt the authors have not indicated the state of hydration and, therefore, the absolute iron content cannot be calculated.

I.V. = intravenous S.C. = subcutaneous

The intravenous toxicity data are a little more difficult to appraise due largely to the paucity of data. The available data indicate one fact with striking clarity: these preparations are considerably more toxic by the intravenous than by the oral route of administration. The intravenous toxicity of ferrous sulfate in mice appears to be approximately 70 mg./kg.[13]. Interestingly, the intravenous toxicity value for ferric chloride appears to be of an order of magnitude similar to that for ferrous sulfate. An intravenous value of 30 mg./kg. of iron or approximately 107 mg./kg. of salt was reported for ferrous chloride in the dog[73].

Following oral administration of toxic doses of ferrous sulfate and related compounds in the mouse and rat, the animal becomes depressed within a few minutes. This depression deepens into complete prostration during which the respiration becomes shallow and rapid. Most of the deaths usually occur within 2 to 6 hours, following a brief terminal convulsive episode. Cessation of respiration precedes cardiac arrest. Those animals which survive invariably show evidence of an intense diarrhea the day following medication. These survivors also exhibit a decreased interest in food for a day or so and frequently there are delayed deaths during the first 2 or 3 days. Toxic symptoms in higher species, such as the cat and dog, appear to be similar except that copious vomiting is produced in contrast to the lower rodent species, where this protective mechanism is absent.

Inspection of the viscera immediately following death reveals the presence of mild to severe congestion of the gastric mucosa even to the point of fresh blood in the stomach, depending upon the dose and concentration of the preparation administered. Hyperemic to petechial hemorrhagic areas may be found in the small intestine. The liver usually shows marked congestion and several to many petechial hemorrhagic areas are usually seen in the lungs. Tissue changes present at death occurring several days after oral medication include marked erosion of the gastric mucosa with fibrotic changes particularly in the greater curvature and antrum, and congestion in the liver, lungs and kidney.

Recently, Nissim[52] called attention to the capillary damaging and anticoagulant effects of various iron preparations and the striking agreement with the incidence of extensive hemorrhages in the lungs with these preparations. Interestingly enough, some 90 years ago, Tourdes[79] observed a "thinning of the blood" in experimental animals suffering from iron intoxication and had suggested that ferrous sulfate may inhibit the coagulation of blood.

CHRONIC TOXICITY OF IRON COMPOUNDS. Studies by Hendrysch and Klimesch[35], using ferrous carbonate, ferrous chloride, and sodium ferricitrate intramuscularly or subcutaneously in rabbits and dogs, showed that administration of small amounts of these iron compounds over periods up to 4 months produces a chronic and sometimes fatal poisoning. These authors concluded that the differential toxicity of iron salts is not based strictly on iron content. Hoff[36] administered small daily doses of ferric chloride (about 300 mg. of iron or about 870 mg. of anhydrous ferric chloride) to a dog in which the liver was by-passed by means of an Eck fistula. "Chronic cerebral intoxication" was reported.

Clinical Toxicity. Ferrous sulfate is the causative agent in the majority of iron poisonings, but fatal ingestion of ferrous chloride, ferric chloride, and ferric ammonium citrate has been reported. In every case, nineteenth century and contemporary, the clinical

aspects have been surprisingly similar. Initially there appear nausea and some vomiting, progressing to severe gastroenteritis with hematemesis, abdominal pain, and diarrhea. Lassitude is followed closely by development of marked shock, usually 4 to 6 hours after ingestion. If the patient survives this collapse, there generally ensues a period of considerable clinical improvement. A second crisis occurs 20 to 50 hours after ingestion of the iron preparation; and if this latter stage of shock, arising from gastric mucosal corrosion, does not terminate fatally, recovery is usually ensured. Hematochezia, convulsions, and motor disturbances are seen occasionally[1,19,31,61,75,79]. Postmortem findings include necrosis of the gastric and intestinal mucosa and congestion or necrosis of the liver. In addition, lung and kidney congestion are frequently observed. Fatal outcome following overdosage with iron varies widely, not only with dose, but also with age, physical condition, and individual susceptibility.

CASE REPORTS ON OVERDOSAGE WITH ORALLY INGESTED IRON PREPARATIONS

A. *Ferrous Sulfate (36.76% iron in anhydrous salt, 20.09% in USP crystalline).* Ferrous sulfate has been the toxic agent in nearly all the reported poisonings, accidental and homicidal. Of the 63 cases with this salt, 23 (two adults and 21 children) ended fatally. In many of the recent instances, the source of iron was "Fersolate," a British proprietary preparation consisting of 200 mg. (3 gr.) of $FeSO_4$, 2.6 mg. (1/25 gr.) of $CuSO_4$, and 2.6 mg. (1/25 gr.) $MnSO_4$ per sugar coated tablet. As few as 15 to 16 of these tablets in a single dose have proved fatal to a 19-month-old child, and 8 are reported to have produced a severe reaction in a child of 2 years. It should be noted that laboratory tests indicate that neither the manganese nor the copper sulfate present contribute materially to the toxic action[25,71]. Obstruction of the stomach occurred in 7 cases. Two instances are considered in detail, one a 3-year-old boy who had ingested about 67 ferrous sulfate tablets[10] and the second, a 17-month-old boy who swallowed 6 to 12 "Fersolate" tablets[62]. Each patient exhibited typical symptoms of ferrous sulfate poisoning so that gastric lavage was performed and anti-shock treatment administered. After several days, both had improved and were vomiting only occasionally. About 3½ weeks after ingestion, emesis increased in frequency and severity. Radiograms made 4 hours after a barium meal showed no barium had left the stomach of either child. In the first case, the stomach was empty 24 hours later, but in the second, only a small amount of barium was observed in the transverse colon after approximately 20 hours. Both children were clinically worse and surgery seemed the best course. Upon operation, thickening and stenosis of the pylorus were found, which were more severe in the case of the younger child. The first patient made a satisfactory recovery, but the second died of acute suppurative peritonitis following the operation.

In both animals and humans who have died after overdoses of iron, hemorrhagic gastritis with edema has been observed in postmortem examination. Both Crosskey and Ross felt that fibrous contracture of the pyloric antrum and pyloric stenosis probably resulted from this persistent intense gastritis.

A summary of fatal cases appears in Table 5 and of nonfatal, in Table 6.

It should be pointed out that in many cases authors have not identified the preparation nor indicated the state of hydration of the ferrous sulfate. Different manufacturers declare in terms of the anhydrous, exsiccated or

U.S.P. (crystalline) salt; some make no indication at all of the state of hydration. Generally, one can assume that the 0.2 gm. tablets are exsiccated ferrous sulfate U.S.P. (approximately 30% iron) and the 0.3 gm. ones are U.S.P. crystalline ferrous sulfate (approximately 20% iron), although this is not invariably the case. Further confusion exists among different official prepara-

TABLE 5.—SUMMARY OF DEATHS DUE TO FERROUS SULFATE

No.	*Year*	*Age*	*Sex*	*Approximate Dose of $FeSO_4$**	*Time of Death after Ingestion*	*Comments*	*Ref.*
1	1850	Child	?	? plus alum	?	Murder. Sentenced to 10 years enforced labor	14
2	1851	Adult	M	? in beef broth	36 hrs.	Murder. Wife condemned to death	8
3	1851	4 yrs.	?	?	?	Murder	54
4	1851	10 mo.	F	50 gm.	36 hrs.	Murder	54, 78
5	1888	5 yrs.	M	648 mg.	24 hrs.	Accident. Intended as an anthelmintic	24
6	1947	3½ yrs.	M	10 gm.	33 hrs.	Accidentally ingested 50 Fersolate tablets	25
7	1947	16 mo.	F	5.2 gm.	21 hrs.	Accident. Source was 26 Ferosolate tablets	76
8	1947	12 mo.	M	6-7 gm.	30 hrs.	Accident. 30 to 35 Fersolate tablets. Treated for shock and aspiration pneumonia	25
9	1948	26 yrs.	M	115 gm.	3 hrs.	About ¼ lb. of U.S.P. ferrous sulphate	27
10	1949	11 mo.	F	?	39 hrs.	Accidentally ingested unknown quantity of Fersolate tablets	57
11	1950	17 mo.	F	6 gm.	11 hrs.	Accident. No more than 20 × 0.3 gm. $FeSO_4$ tablets. Methylene blue gave temporary improvement.	67
12	1951	12 mo.	M	?	4½ hrs.	Accident. Unknown number of $FeSO_4$ tablets. Only medical treatment consisted of castor oil	72
13	1951	19 mo.	F	3.0-3.2 gm.	4¼ hrs.	Accident. 15 to 16 $FeSO_4$ tablets. Two hospitals refused admission. Doctor prescribed orange juice	72
14	1951	18 mo.	M	8.8 gm.	5½ hrs.	Accident. 44 $FeSO_4$ tablets. Stomach lavaged. Restoratives given	72
15	1951	14 mo.	F	8 gm.	20 to 24 hrs.	Accident. 44 $FeSO_4$ tablets. Returned home. Doctor felt no danger, prescribed castor oil and kaolin	72
16	1952	26 mo.	F	9 to 12 gm.	4½ hrs.	Accident. 30 to 40 × 0.3 gm. chocolate coated tablets. Gastric lavage plus supportive therapy	12
17	1952	19 mo.	M	?	40 hrs.	Accident. Unknown number enteric coated 0.2 gm. tablets. Gastric lavage, supportive therapy, antibiotics, BAL without improvement	75
18	1952	21 mo.	M	8.2 gm.	4 hrs.	Accident. About 41 Fersolate tablets. Gastric lavage with sodium bicarbonate	66
19	1952	17 mo.	M	?	?	Accident. Unknown quantity of tablets	80
20	1952	2 yrs.	M	13.8 gm.	7 hrs.	Accident. About 43 × 0.32 gm. $FeSO_4$ tablets	80
21	1953	29 mo.	M	22.5 gm.	4½ hrs.	Accident. 75 × 0.3 gm. tablets. Gastric lavage	4
22	1954	20 mo.	F	10.2 to 14.2 gm.	20¾ hrs.	Accident. 34 to 44 × 0.3 gm. enteric coated $FeSO_4$. Supportive therapy	9
23	1954	21 mo.	F	?	48 hrs.	Accident. ? × $FeSO_4$ exsic. 0.162 gm. + liver conc. NF. Supportive therapy	11

NOTE. Cases 1 to 5 were probably $FeSO_4 \cdot 7H_2O$ but authors do not so indicate. Cases 12 to 15 were probably Fersolate.

* Based on each author's report. No attempt has been made to convert U.S.P. crystalline ferrous sulfate ($FeSO_4 \cdot 7H_2O$). [See text.]

TABLE 6.—NONFATAL POISONING FROM FERROUS SULFATE

No.	*Year*	*Age*	*Sex*	*Approximate Dose of $FeSO_4$**	*Length of Convalescence*	*Comments*	*Ref*
1	1850	22 yrs.	F	?	?	Attempted murder. Husband sentenced to 5 yrs. Nearly fatal. Commercial green vitriol	[illegible]
2	1859	36 yrs.	M	? gm. in wine	3 days	Attempted murder. Commercial ferrous sulfate. Seriously ill	[illegible]
3	1881	17 yrs.	M	?	..	Attempted murder. Commercial ferrous sulfate. Small amount of cornmeal. Slightly ill.	[illegible]
4	1881	12 yrs.	F	?	Several days	Sister of Case 3. Poisoned on same occasion	[illegible]
5	1881	45 yrs.	F	?	2 weeks	Mother of Cases 3 and 4. Poisoned on same occasion	[illegible]
6	1881	70 yrs.	M	?	2 days	Father of Cases 3 and 4. Poisoned on same occasion	[illegible]
7	1883	40 yrs.	F	56 gm.	3 mo.	Attempted suicide. Stormy course for more than 2 mos.	[illegible]
8	1934	Child	F	28 gm.	?	No details given	25, [illegible]
9	1936	30 yrs.	M	32.4 gm. in 26 days	?	3 × 0.375 gm. per day. Anemia therapy. Epileptiform seizures. Patient weighed 86.5 lbs.	[illegible]
10	1936	Adult	F	24.75 gm. in 33 days	?	0.75 gm./day. Anemia therapy. Epileptiform seizures	[illegible]
11	1947	2 yrs.	M	1.6 gm.	15 days	Accident. 10 Fersolate tablets. Returned 2. Emetics and supportive therapy	[illegible]
12	1949	16 mo.	M	6 gm.	1 week	Accident. About 50 Fersolate tablets. Returned 20. Gastric lavage, supportive therapy and BAL	[illegible]
13	1950	4½ yrs.	F	0.8 gm.		Accident. 24 Fersolate tablets. Returned 20. Received 0.15 gm. $NaHCO_3$ every 4 hrs.	[illegible]
14	1950	19 mo.	M	2 gm.	..	Accident. About 10 Fersolate tablets. Gastric lavage with $NaHCO_3$; BAL	[illegible]
15	1950	2½ yrs.	M	2 to 4 gm.		Accident. 10 to 20 Fersolate tablets. Given syrup of figs	[illegible]
16	1951	14 mo.	?	4 gm.		Accident. 19 to 20 $FeSO_4$ tablets	[illegible]
17	1951	2½ yrs.	F	?	3 days	Accident. About 60 $FeSO_4$ tablets [illegible] returned "nearly all"	[illegible]
18	1951	21 mo.	F	10.8 gm.	26 days	Accident. About 75 $FeSO_4$ tablets [illegible] returned 21. Gastric lavage	[illegible]
19	1951	23 mo.	M	6.5 gm.	3 weeks	Accident. 16 $FeSO_4$ tablets and 10 iron "plastules." Returned 4 tablets and partly dissolved "plastules"	[illegible]
20	1951	11 mo.	M	1.4 to 1.8 gm.	3 days	Accident. 13 $FeSO_4$ tablets but returned pieces = to 4 to 6 tablets. Gastric lavage	[illegible]
21	1951	20 mo.	M	0.6 gm.	3 hrs.	Accident. About 5 $FeSO_4$ tablets but returned 2. Ill enough to hospitalize	[illegible]
22	1951	30 mo.	F	15 gm.	11 days	Accident. Believe about 75 × 0.2 gm. $FeSO_4$ tablets. Gastric lavage. $NaHCO_3$. Penicillin	[illegible]
23	1952	15 mo.	F	4.5 to 6 gm.	54 hrs.	Accident. 15 to 20 × 0.3 gm. $FeSO_4$ tablets. Tablet fragments returned	[illegible]
24	1952	18 mo.	M	4.5 gm.	7 days	Accident. 15 × 0.3 gm. $FeSO_4$ tablets. Gastric lavage, plasma, penicillin	[illegible]
25	1952	3 yrs.	M	?	2½ mo.	Accident. 67 × ? gm. $FeSO_4$. Gastric lavage, nikethamide, and methionine. Pyloric stenosis necessitated surgery	[illegible]
26	1952	19 mo.	F	?	1 week	Accident. 10 × ? $FeSO_4$ tablets. Gastric lavage and supportive therapy	[illegible]

TABLE 6.—NONFATAL POISONING FROM FERROUS SULFATE—(*Continued*)

No.	*Year*	*Age*	*Sex*	*Approximate Dose of $FeSO_4$**	*Length of Convalescence*	*Comments*	*Ref.*
27	1953	17 mo.	M	1.2 to 2.4 gm.	Died of peritonitis following surgery	Accident. 6 to 12 Fersolate tablets. Gastric lavage and antishock treatment. Pyloric stenosis necessitated surgery twice	62
28	1954	14 mo.	F	15 to 22.5 gm.	10 days	Accident. 50 to 75 × 5 gr. $FeSO_4$ tablets. Gastric lavage and BAL	65
29	1954	21 mo.	F	?	8 weeks	Accident. Unknown number of Fersolate. Pyloric stenosis requiring surgery	81
30	1954	2 yrs.	M	8 gm.	1 mo.	Accident. 40 Fersolate. $NaHCO_3$ lavage returned broken tablets. Pyloric stenosis treated surgically	81
31	1954	20 mo.	F	13 gm.	6 days	Accident, 65 capsules × 0.2 gm. $FeSO_4$, 3.25 mg. molybdenum oxide. Gastric lavage. I.V. $NaHCO_3$ and BAL	3
32	1954	16 mo.	F	?	8 weeks	Accident. ? × Ferrous Sulfate tablets. Vomited 20. $NaHCO_3$ lavage. Pyloric stenosis required surgery	23
33	1954	15 mo.	F	2.4 gm.	4 days	Accident. 8 × 0.3 gm. $FeSO_4$ tablets, enteric-coated	9
34	1954	13 mo.	F	3.8 to 5.7 gm.	8 days	Accident. 20 to 30 × 0.19 gm. $FeSO_4$ tablets. $NaHCO_3$ lavage. Supportive therapy	42
35	1954	17 mo.	F	2 to 3 gm.	3½ mo.	Accident. 10 to 15 Fersolate. Supportive therapy. Pyloric obstruction required surgery	26
36	1954	13 mo.	M	?	2 mo.	Accident. ? × Fersolate. $NaHCO_3$ lavage. Supportive therapy. Pyloric obstruction treated surgically	26

NOTE: No details available on 4 other nonfatal cases.[26] Cases 17 to 21 are probably Fersolate.

* Based on each author's report. No attempt has been made to convert to U.S.P. crystalline Ferrous Sulfate ($FeSO_4 \cdot 7H_2O$). [See text.]

tions of the exsiccated form, the U.S.P. material containing not less than 80% anhydrous salt, $FeSO_4$ exsic. B.P. not less than 77%, and official material in Norway 80.5 to 85%. "Fersolate," for instance, declares 0.2 gm. exsic. $FeSO_4$ (at least 77% anhydrous salt equivalent to about 29% ferrous iron); but a publication from the manufacturer's research laboratories reports the iron content as 24%[71]. Thus, actual dose in terms of anhydrous or U.S.P. crystalline ferrous sulfate or ferrous iron content frequently cannot be determined with any accuracy.

In some instances (Table 5, Nos. 12, 13, 15), the children remained at home with little or no medical care beyond castor oil and reassurance. Lack of appreciation of the reality of ferrous sulfate poisoning by doctors and hospitals makes it necessary to emphasize that ferrous sulfate intoxication may be serious, and that immediate treatment is essential[12,17].

B. *Ferrous Chloride (44.06% iron in anhydrous salt, 28.09% in crystalline tetrahydrate).* The use of ferrous chloride in Sweden has resulted in at least 3 cases of iron toxicity. A 2½-year-old girl swallowed about 20 tablets, each containing 0.267 gm. of ferrous chloride (5.34 gm.). The child exhibited typical symptoms of iron poisoning, but

survived. Some residual signs of stomach damage were still evident by roentgenogram 6½ months after ingestion of the tablets and the child's general condition continued poor for a considerable time[45]. In the same report, Lindquist noted a second case of very severe ferrous chloride poisoning following ingestion of about 40 tablets of 0.267 gm. of $FeCl_2$. Extensive necrosis through all the layers of the stomach wall was observed.

More recently a third case, that of a 17-month-old boy, has been reported[56]. The child, while playing with its mother's anti-anemia iron tablets, swallowed an unknown number. No symptoms developed till 4 hours later, and within one-half hour his condition was serious enough to require hospitalization. Methylene blue plus intravenous fluids brought about a decided improvement, but the child's condition once more began to deteriorate so that, in spite of continued therapy, he expired 28 hours after ingestion of the tablets.

Lindquist suggests that the assertion in a pharmacology text that ferrous chloride had no caustic effect and that overdosage involved no risk, was probably based on the paucity of reported cases from ferrous chloride. However, on the basis of the cases reported, he concluded that ferrous chloride, like ferrous sulfate, may prove very dangerous, at least to children.

C. *Ferrous Gluconate* (*12.52% iron in anhydrous salt, 11.58% in dihydrate*). Ferrous gluconate, since the work of Reznikoff and Goebel[60,61] reported in 1937, has become increasingly popular as a source of iron in anemia therapy. It is the most readily absorbed of all ferrous salts[33] and has been found to produce less gastric upset[33,60,61]. Holly[37] recently reported on the administration of ferrous gluconate to pregnant, nonpregnant, anemic and normal females. Patients received as much as 1 gm. per day for up to 76 days without indication of ill effects.

Toxic symptoms are apparently exceedingly rare beyond occasional nausea, and the like, in susceptible individuals, and even here symptoms are less severe than with other forms of iron[37,60]. No reports have been found in the literature of poisoning from this salt, and the medical files of a major producer of this preparation contain no privately reported cases of reactions following overdosages in humans[2]. Further, an English source has indicated recently that 8 ferrous gluconate (Fergon) tablets were ingested, without any untoward effects, by an 11-month-old girl[2]. Clinically, at least, this iron salt appears less irritating and less toxic than other common sources of iron.

D. *Ferric Ammonium Citrate* (*14.5 to 16% iron in the green salt, 16.5 to 18% in the brown form*). Ferric ammonium citrate has become a common medicinal form of iron, since it has been found to be utilized by the body and is lacking in the objectionable astringent properties found in simple ferric salts[5]. However, this salt is not without toxic effects.

In 1949, a 26-year-old pregnant woman took a mixture of 15 gm. of iron ammonium citrate in whiskey, apparently in the hope of inducing an abortion. She died 3 days later of toxic hepatitis[15]. Hurst[11], in 1931, reported a case of iron encephalopathy resulting from iron ammonium citrate. A 58-year-old woman, suffering from anemia while in the hospital, received 4 × 40 gr. (10 gm.) of the iron salt per day for 23 days. On the 24th day, the daily dose was increased to 2 × 40 gr. plus 2 × 60 gr. (12.5 gm.). Nine times the following morning the patient, while vomiting, lost consciousness. Her breathing became stertorous, the face

was cyanosed, eyes deviated to the right, and pupils dilated. Plantar reflexes were extensor. Between attacks, the patient was semi-conscious and appeared to have a headache. Iron therapy was stopped and the patient gradually recovered.

E. *Ferric Chloride (34.43% iron in anhydrous salt, 20.66% in hexahydrate).* Ferric chloride was the toxic agent employed in 4 homicides reported by Peterson *et al.*[55]. As little as 1½ oz. (45 cc.) of tincture of ferric chloride (6 gm. of salt) proved fatal to an adult male when taken internally. However, in 4 cases involving 1 to 3 oz. of this tincture, the women survived in each case[65]. Ravaglia, in 1884[59], recorded a case of attempted murder with ferric chloride. The woman survived but was troubled by a general dyspeptic disturbance for one month.

Human Lethal Dose. Any attempt to estimate the human fatal dose of these preparations must be made in full recognition of the inherent errors involved. The data are collected from accidental poisoning cases in which it is often difficult or impossible to establish with accuracy the amount consumed. Even though the error may be large, it becomes of interest to examine the summary of the case reports of deaths due to ferrous sulfate as given in Table 5. In several instances reasonably accurate information was available on the amounts of ferrous sulfate ingested. It is possible to make a rough approximation of the fatal dose in terms of mg./kg. of ferrous sulfate for some of the cases.

Death has occurred from the oral ingestion of ferrous sulfate at dosages ranging from 40 to 1600 mg./kg., with an average value of approximately 900 mg./kg. of ferrous sulfate. Comparing this value with those found for the fatal dose of ferrous sulfate in experimental animals it is apparent that this is considerably smaller than the acute oral toxicity value given for the mouse (4500 mg./kg.), guinea pig (1500 mg./kg.) and rabbit (3000 mg./kg.) and of approximately the same magnitude as that given for the cat (>500 mg./kg.) and the dog (800 mg./kg.). It will be noted, of course, that the figure, 900 mg./kg., is based largely on ferrous sulfate poisoning in cases approximately 2 years old and younger. Further consideration of the relative toxicity of ferrous sulfate in man and animals will be taken up again in the presentation of the experimental data from this laboratory[38].

Probable Toxicity Mechanisms. Various mechanisms have been postulated in an effort to explain the cause of death in cases of poisoning following the oral ingestion of iron salts[18,43,58,66,72]. It is difficult to establish that any one factor is solely responsible. Mounting evidence tends to bring into focus the role of the gastrointestinal irritation observed following the fatal ingestion of these preparations. The severe nausea, hematemesis, abdominal cramps and diarrhea followed by the development of profound shock all tend to point to the potentially corrosive effects of these salts as a starting point in the chain of events which leads to a fatal outcome. It has been suggested that the initial effect is a direct corrosion of the gastric mucosa which results in excessive absorption of iron into the systemic circulation with the formation of apoferritin. This then combines with the iron to form ferritin[66], the substance thought to be identical with the vasodepressor material (V.D.M.) found in the blood of animals in experimentally induced shock.

Although vomiting does occur in the human, it does not seem especially reliable as a protective mechanism in iron poisoning. Particularly in young children, the rapidly developing tissue

destruction following the ingestion of large amounts of ferrous salts appears to interfere with these efforts to rid the stomach of massive quantities of iron, often with fatal results. This factor should tend to emphasize the importance of *prompt* and *gentle* gastric lavage combined with vigorous supportive therapy for shock in suspected poison cases. Further, it should stimulate the search for less irritant forms of iron for oral medicinal use.

Summary. The literature on the toxic effects of iron compounds in man and animals is reviewed. The oral median lethal dose in different species has been approximated from published data for the common iron salts. In addition, an estimated fatal dose for humans has been calculated from cases of ferrous sulfate poisoning. Probable mechanisms of toxicity are discussed.

Conclusions. 1. There are adequate data in the literature to establish conclusively that iron salts are toxic to both man and animals. Of 78 cases of poisoning reported in man, 30 ended fatally.

2. The oral toxicity of iron compounds is not a function of the iron content alone, but is dependent upon the particular salt as well.

3. The majority of reported poisonings in man are due to ferrous sulfate. Of the 63 cases reported as due to this salt, 23, or more than one-third, ended fatally. From these data the fatal dose of ferrous sulfate in humans is estimated to be approximately 900 mg./kg.

4. A smaller number of cases of poisoning have been reported after the ingestion of ferrous chloride, ferric chloride and ferric ammonium citrate.

5. No cases of poisoning have been reported from ingestion of ferrous gluconate.

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AN EXPERIMENTAL STUDY OF THE TOXICITY OF FERROUS GLUCONATE

By James O. Hoppe, Ph.D.
MEMBER

G. Marie Agnew Marcelli, M.S.
RESEARCH ASSOCIATE

AND

Maurice L. Tainter, M.D.
DIRECTOR

(From the Sterling-Winthrop Research Institute, Rensselaer, New York)

From an extensive survey of the pharmacological and clinical literature on the toxicity of iron[3] it appeared that ferrous gluconate was less dangerous in overdoses than the other popular iron salts. No cases of poisoning from this iron salt have appeared in the literature, in contrast to the numerous ones with other iron compounds, and particularly with ferrous sulfate. Few pharmacologic data have been published on ferrous gluconate. Therefore, it was decided to carry out a series of studies in our laboratories which would explore and more accurately define this apparent lower experimental and clinical toxicity. Observations on the systemic and local toxicity of ferrous sulfate were included for comparison with the ferrous gluconate results.

Methods. The acute toxicity of ferrous gluconate was determined in direct comparison with that of ferrous sulfate following both intravenous and oral administration in male, albino Swiss mice weighing 22 ± 2 gm. For the intravenous injection, the compounds were administered in aqueous solution in a volume of 0.01 cc./gm. of body weight at a rate of 1.0 cc./minute. A volume of 0.01 cc./gm. of body weight also was used for oral administration. In addition, the acute toxicity of ferrous gluconate was compared with that of ferrous sulfate following oral administration in male, Sprague-Dawley rats weighing 100 ± 10 gms. The compounds in aqueous solution were administered orally in a volume of 1.0 cc./100 gm. of body weight. The mice and rats were observed closely for several hours following injection, and the LD_{50} ± its standard error was estimated at the end of 24 hours by the method of Miller and Tainter[4]. The animals were held under close observation for a period of one week following injection and any delayed manifestations of toxicity were recorded. Where delayed deaths occurred after 24 hours, the LD_{50} was recalculated at the end of the 7-day observation period. Ferrous gluconate and ferrous sulfate were administered orally as a finely divided powder by capsule to cats, weighing 2 to 3 kg., and to mongrel dogs, weighing 7 to 12 kg., in an effort to determine the acute lethal dose following oral administration. After failing to produce fatalities by oral administration of large single doses of either compound, an effort was made to determine whether death occurred following repeated medication with massive oral dosages. Daily doses of 25, 50, 100, 200 and 400 mg./kg. of ferrous sulfate and 100, 200, 400, 800 and 1600 mg./kg. of ferrous gluconate were administered as a powder by capsule to two cats at each dose level 5 days a week for 2 weeks. The cats were observed closely following each medication for evidence of systemic intoxication and the body weights were recorded 3 times a week. All animals were housed in air-conditioned quarters with food and water available at all times, with the exception of the period immediately preceding the oral medications. The mice and rats were fasted for 4 hours and the cats and dogs for 18 hours before oral administration of the ferrous gluconate and ferrous sulfate dosages.

Local tissue toxicity was estimated by means of the trypan blue irritation test procedure[4]. Saline or aqueous-saline solutions of ferrous gluconate from 1% to 8% and ferrous sulfate from 0.25% to 2%, were injected intracutaneously into the abdominal skin of the rabbit followed by the intravenous injection of 10 mg./kg. of trypan blue. The results are expressed in terms of the Threshold Irritant Concentration (TIC) or that concentration, in per cent, which produces no more than a mild irritation (a faint but discernible blue color at the site of injection).

Ferrous gluconate* and ferrous sulfate, U.S.P., were administered as the salt in each case. The results have been calculated in terms of iron in order to provide a more direct comparison of the toxicity values. Percentage factors used for these calculations were as follows:

Ferrous sulfate .7 H_2O = 20.09% iron
Ferrous gluconate .2 H_2O = 11.58% iron

to 5 days. No deaths occurred after 5 days. The LD_{50} value for ferrous gluconate at 7 days was not significantly different from the 24-hour value. The 7-day LD_{50} value for ferrous sulfate, however, indicated a significant increase in toxicity due to delayed deaths. In the acute deaths, the mice were severely depressed and lapsed into complete prostration which terminated in a brief clonic convulsive episode with cessation of respiration preceding cardiac arrest. A majority of the acute deaths occurred in one to five minutes after intravenous injection.

b. *Oral.* The acute oral toxicity data in Table 1 show that ferrous gluconate is significantly less toxic than ferrous

TABLE 1.—ACUTE TOXICITY OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) VERSUS FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) IN MICE

Compound	Route of Admins.	No. of Animals	$LD_{50} \pm$ s.e. mg./kg.			
			As Salt		As Fe^{++}	
			24 Hours	7 Days	24 Hours	7 Days
Ferrous sulfate	I.V.	30	65 ± 4.8	51 ± 4.6	13 ± 1	10.2 ± 0.9
Ferrous gluconate	I.V.	40	114 ± 7.6	98 ± 6.8	12.5 ± 0.7	10.8 ± 0.7
Ferrous sulfate	Oral	30	1520 ± 130	1520 ± 130	306 ± 26	306 ± 26
Ferrous gluconate	Oral	60	3700 ± 145	3700 ± 145	429 ± 17	429 ± 17

1. ACUTE TOXICITY STUDIES IN THE MOUSE. a. *Intravenous.* As shown in Table 1, ferrous sulfate was found to be approximately twice as toxic as ferrous gluconate in terms of the salt. When the data were calculated in terms of ferrous iron, there did not appear to be any apparent difference in the acute intravenous toxicity of these two compounds in mice. The value of 13 ± 1 mg./kg. for ferrous sulfate is in almost precise agreement with the value, 13.8 mg./kg. of iron, reported for ferrous sulfate in mice by Edge and Somers[1].

Although a majority of the mice died in the first 24 hours following injection, several deaths occurred in the next 3

sulfate, both in terms of the salt and of ferrous iron. The oral LD_{50} for ferrous sulfate was found to be 1520 mg./kg. compared with 3700 mg./kg. of ferrous gluconate which, when expressed in terms of ferrous iron, amounts to 306 mg./kg. as the sulfate and 429 mg./kg. as the gluconate. These differences are statistically significant and indicate that the gluconate, in terms of ferrous iron content, is approximately 40% better tolerated than the sulfate. There were no delayed deaths with either compound following oral administration in mice.

2. ACUTE ORAL TOXICITY STUDIES IN THE RAT. The acute oral toxicity data

*Ferrous gluconate was used in the form of Fergon, supplied by Winthrop-Stearns Inc.

in the rat were found to be of a similar order of magnitude as those found in the mouse as will be noted from the data in Table 2. In terms of the salts, ferrous gluconate was found to be approximately one-third as toxic as ferrous sulfate following oral administration in the rat. When compared in terms of ferrous iron, ferrous gluconate is significantly less toxic, being approximately one-half as toxic as ferrous sulfate. No delayed deaths were observed with ferrous sulfate; one delayed death was observed with ferrous gluconate.

3. ACUTE ORAL TOXICITY IN THE CAT. It was not possible to obtain mortality data by this route of administration at the dose levels employed, since emesis occurred in every cat within 15 minutes to one hour after medication. Severe diarrhea also was observed but became less evident at the higher dosages as the promptness and intensity of emesis increased. It was concluded from these experiments that the acute oral lethal dose of ferrous sulfate in cats was more than 200 mg./kg. and more than 400 mg./kg. for ferrous gluconate.

The pattern of emesis was sufficiently prominent and consistent to permit the estimation of the approximate median emetic dose, AED_{50}, (the approximate dose producing emesis in 50% of the cats) as a criterion for comparing the gastric tolerance to these two compounds in cats. A summary of the emetic effects and of the incidence of diarrhea is given in Table 3. It will be noted that the dose of ferrous gluconate required to produce emesis in 50% of the cats was more than three times as large as that of ferrous sulfate. About twice as much iron in the form of ferrous gluconate was tolerated without vomiting as was tolerated in the form of the sulfate.

4. ACUTE ORAL TOXICITY STUDIES IN THE DOG. Six dogs, one at each dosage level, were given capsules of finely divided ferrous gluconate in amounts ranging from 100 to 3200 mg./kg. The other

TABLE 2.—ACUTE ORAL TOXICITY OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) AND FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) IN RATS

Compound	No. of Animals	$LD_{50} \pm$ s.e. mg./kg.			
		As Salt		As Fe^{++}	
		24 Hours	7 Days	24 Hours	7 Days
Ferrous sulfate	30	1480 ± 184	1480 ± 184	298 ± 37	298 ± 37
Ferrous gluconate	30	4600 ± 560	4500 ± 400	518 ± 63	507 ± 45

TABLE 3.—EFFECTS OF SINGLE ORAL DOSAGES OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) AND FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) IN CATS

Compound	Dose mg./kg.	Emetic Effects			Diarrhea
		No. Vomited / No. Medicated	AED_{50}, mg./kg.		No. Showing Diar. / No. Medicated
			As Salt	As Fe^{++}	
Ferrous sulfate	25	1/4	82	16	2/4
	50	2/4	..	..	2/4
	100	1/4	..	..	0/4
	200	4/4	..	..	0/4
Ferrous gluconate	100	0/4	267	31	2/4
	200	1/4	..	..	1/4
	400	4/4	..	..	0/4

dogs received similar capsules of ferrous sulfate in doses from 50 to 800 mg./kg. No deaths or serious evidence of acute systemic intoxication were observed in the dogs at doses up to and including the highest dose level, 800 mg./kg. of ferrous sulfate or 3200 mg./kg. of ferrous gluconate. The most obvious effects produced by these two compounds were emesis and diarrhea (Table 4). Vomiting was noted in the dog receiving 50 mg./kg. of ferrous sulfate but was not encountered in the others until the dose was raised to 800 mg./kg., when a prompt and vigorous emetic reaction was observed. With ferrous gluconate, vomiting did not occur until doses of 1600 and 3200 mg./kg. were reached. A watery diarrhea became apparent approximately one hour after oral administration of 100 mg./kg. of ferrous sulfate and 800 mg./kg. of ferrous gluconate. At doses of 200 and 400 mg./kg. of ferrous gluconate, diarrhea developed the morning of the day following medication.

The occurrence of vomiting and diarrhea, indicative of a protective mechanism similar to that observed in the cat, interfered with the attempt to estimate the acute oral lethal dosage of these compounds in dogs.

5. REPEATED ORAL MEDICATION IN THE CAT. Since it had not been possible to obtain mortality following oral administration of large single doses of either compound in the cat, an effort was made to determine whether death would result from repeated medication with massive hypertherapeutic doses. Daily doses of 25, 50, 100, 200 and 400 mg./kg. of ferrous sulfate and 100, 200, 400, 800 and 1600 mg./kg. of ferrous gluconate were administered as a powder by capsule to 2 cats at each dose level 5 days a week for 2 weeks. No serious body weight changes or mortality occurred among the cats receiving ferrous gluconate. However, one cat on 400 mg./kg. of ferrous sulfate died following the fifth dose. Some impairment of appetite occurred in the second cat at this dose level, but no serious loss in weight occurred and the cat survived the full medication schedule. Occasional vomiting and diarrhea occurred at the lower dosages with both compounds as noted in Table 5. The intensity of the emesis increased with increase of dosage and was associated with a decrease in the incidence of diarrhea. The emesis appeared to be entirely local in effect, since it occurred in less than an hour after medication. Other than the emesis, the cats appeared to suffer no ill effects from the medication. The appetite except at the highest dosages remained normal in every cat.

6. TISSUE IRRITATION STUDIES. Because of the apparent difference in incidence of gastrointestinal irritation observed with these two compounds in cats and dogs, a comparison of their irritant properties was made by means of the trypan blue irritation test[2] with results as summarized in Table 6.

Ferrous gluconate was observed to be distinctly less irritant than ferrous sulfate. The relative irritancy of these two compounds was similar to that observed in the acute oral studies in cats. The TIC (threshold irritation concentration) for ferrous sulfate was found to be 0.25% and for ferrous gluconate four times larger or 1.0%. Recalculation of these data in terms of ferrous iron indicates that the local tissue irritation of ferrous gluconate is less than one-half that of ferrous sulfate. The evidence of a lower local tissue toxicity with ferrous gluconate correlates well with the finding that the acute oral toxicity of ferrous gluconate is significantly less than that of ferrous sulfate upon oral administration to the mouse and rat. In addition, these laboratory results confirm the clinical observations that

ferrous gluconate, being less irritating, is much better tolerated than ferrous sulfate.

Discussion. Comparison of the present acute toxicity data on ferrous gluconate and ferrous sulfate with the data available in the literature indicates some agreement and also some wide discrepancies. The present acute oral LD_{50} values of 1520 ± 130 mg./kg. for ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and 3700 ± 145 mg./kg. for ferrous gluconate ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) in the mouse indicate a higher acute oral toxicity for these substances than that reported for the mouse in the literature.

TABLE 4.—EFFECTS OF SINGLE ORAL DOSAGES OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) AND FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) IN DOGS

	Dose, mg./kg.			
Compound	*As Salt*	*As Fe^{++}*	*Vomiting*	*Diarrhea*
Ferrous sulfate	50	10.0	Yes	No
	100	20.1	No	Yes at 2 hours
	200	40.2	No	Yes at 1 hour
	400	80.4	No	Yes at 1 hour
	800	160.8	Yes at 10 min.	Yes at 1½ hours
Ferrous gluconate	100	11.6	No	No
	200	23.2	No	Yes at 24 hours
	400	46.4	No	Yes at 24 hours
	800	92.8	No	Yes at 1½ hours
	1600	185.6	Yes at 1 hour	Yes at 2 hours
	3200	371.2	Yes at 1½ hours	Yes at 1 hour

TABLE 5.—EFFECTS OF REPEATED MASSIVE ORAL DOSAGE (5 DAYS A WEEK FOR 2 WEEKS) OF FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) AND FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) IN CATS

	Dose, mg./kg.				
Compound	*As Salt*	*As Fe^{++}*	*Mortality*	*Emetic Effects*	*Diarrhea*
Ferrous sulfate	25	5.0	0/2	Occasional, one cat	None
	50	10.0	0/2	Occasional, one cat	Occasional, both cats
	100	20.1	0/2	Frequent, both cats	Occasional, both cats
	200	40.2	0/2	Frequent, both cats	Frequent, both cats
	400	80.4	1/2 (7th day)	Daily	None
Ferrous gluconate	100	11.6	0/2	Occasional, one cat	Occasional, one cat
	200	23.2	0/2	Occasional, both cats	Frequent, both cats
	400	46.4	0/2	Frequent, both cats	Occasional, both cats
	800	92.8	0/2	Frequent, both cats	Occasional, both cats
	1600	185.6	0/2	Daily	Occasional, both cats

TABLE 6.—TRYPAN BLUE IRRITATION DATA ON FERROUS SULFATE ($FeSO_4 \cdot 7H_2O$) VERSUS FERROUS GLUCONATE ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$)

Compound	*Concentration in Per Cent (as salt)*	*Maximum Av. Irritation Score*	*Adjective Rating*	**TIC, %*	
				Salt	*Fe^{++}*
Ferrous sulfate	0.25	1.3	Mild	0.25	0.05
	0.5	7.3	Moderate		
	1.0	16.0	Marked		
	2.0	16.0	Marked		
Ferrous gluconate	1.0	3.3	Mild	1.0	0.12
	2.0	5.3	Moderate		
	4.0	13.3	Marked		
	8.0	16.0	Marked		

* TIC = Threshold irritant concentration.

These variations may be due to differences in methods of administration, strain of mice, conditions of assay, and the like. The acute intravenous toxicity for ferrous sulfate as ferrous iron, 13 ± 1 mg./kg., however, agrees almost precisely with the reported literature value of 13.8 mg./kg.[1] In the case of the cat, a literature value of greater than 500 mg./kg. of ferrous sulfate was reported. In the present study, no mortality was observed with single oral doses of ferrous sulfate up to and including 200 mg./kg. When given by repeated oral administration, however, one of two cats died at the end of the first week at a dose of 400 mg./kg. of ferrous sulfate. An estimated acute oral lethal dose of 800 mg./kg. of ferrous sulfate for the dog has been reported[5]. In the present study no mortality was observed following oral administration of ferrous sulfate at dosages up to and including 800 mg./kg. in the dog. Copious vomiting was encountered in both the cat and the dog, which tended to interfere with attempts to estimate the acute oral lethal dose of ferrous sulfate in these two species.

The fact that copious and effective emesis interfered with the estimation of the acute oral lethal dose of ferrous sulfate in both the cat and dog indicates that this protective mechanism may be better developed in these two species than it is in the human. It is of interest to note that the estimated oral median lethal dose of 900 mg./kg. for ferrous sulfate in children, referred to earlier[5], is within the limits of experimental error for the acute oral LD_{50} values for ferrous sulfate in the mouse (1520 ± 130 mg./kg.) and the rat (1480 ± 184 mg./kg.) as established in the present investigation.

Summary. The results of a direct comparison of the acute systemic and local toxicity of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and ferrous gluconate ($Fe[C_6H_{11}O_7]_2 \cdot 2H_2O$) in experimental animals may be summarized as follows:

1. Studies in mice indicate that the acute intravenous toxicity of ferrous gluconate (114 ± 7.6 mg./kg.) is approximately half that of ferrous sulfate (65 ± 4.8 mg./kg.) in terms of absolute weights of the salts. In terms of iron, however, there is no apparent difference in the toxicity of the two compounds by this route of administration. Delayed deaths occurred with both compounds but were significantly greater with ferrous sulfate.

2. Lower toxicity was observed with both compounds when given orally to mice. The acute oral toxicity values (ferrous sulfate, 1520 ± 130 mg./kg.; ferrous gluconate, 3700 ± 145 mg./kg.) were more than twenty times as large as those following acute intravenous injection. In the rat ferrous gluconate (4600 ± 560 mg./kg.) was only one-third as toxic as ferrous sulfate (1480 ± 184 mg./kg.) as the salt and one-half as toxic in terms of ferrous iron. No delayed deaths of significance were observed following oral administration in either species.

3. Attempts to estimate the acute oral toxicity in cats were unsuccessful, due to intense local gastric irritation which resulted in prompt and copious vomiting. Approximately twice as much ferrous iron in the form of ferrous gluconate as ferrous sulfate was tolerated before vomiting occurred.

4. In the dog the acute oral median lethal dose was estimated to be greater than 800 mg./kg. of ferrous sulfate and more than 3200 mg./kg. of ferrous gluconate. No deaths or serious evidence of acute systemic intoxication were observed at these doses. The emesis and diarrhea produced by both compounds rendered attempts to estimate accurate LD_{50} values impracticable.

5. Local tissue irritation studies indicated that twice as much iron in the

form of ferrous gluconate could be intracutaneously injected without serious damage as could be tolerated in the form of the sulfate.

6. Daily oral administration of ferrous gluconate powder by capsule to cats, 5 days a week for 2 weeks at the hypertherapeutic dosages of 100 to 1600 mg./kg. produced no mortality and no evidence of cumulative toxicity. Emesis and diarrhea were noted at all dose levels. Emesis was particularly prompt and copious at the highest dose levels.

7. Similar daily oral administration of ferrous sulfate to cats at doses of 25 to 400 mg./kg. resulted in death of one of two cats at the 400 mg. level at the end of the first week. Other than emesis and diarrhea, no additional serious toxic effects were noted. No cumulative toxic effects were observed.

8. The magnitude of the acute oral toxicity values when compared with the acute intravenous figures in mice indicates a relatively low order of absorption from the intestinal tract. An additional safety factor is evident from the oral studies in the cat and the dog in which the local irritant effects induce a protective emesis. These data suggest prompt, gentle gastric lavage along with supportive therapy for shock as an effective emergency measure in those cases where, for any reason, vomiting does not occur spontaneously following oral ingestion of ferrus sulfate, ferrous gluconate or other soluble iron salts.

9. These studies clearly establish that ferrous gluconate is less irritating and less toxic than ferrous sulfate when considered from the standpoint of the total weight of drug administered or in terms of their iron contents. A firm experimental basis for the lack of clinical toxicity and for the therapeutic preference for ferrous gluconate, therefore, appears to be demonstrable.

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A PHARMACOLOGICAL INVESTIGATION OF ACUTE IRON POISONING AND ITS TREATMENT

C. S. HOSKING[1]

From the Department of Pathology, University of Queensland

SYNOPSIS

Acute iron poisoning is one of the commoner causes of death in childhood from accidental overdose of therapeutic agents (Westlin, 1966).

This investigation was undertaken to establish some of the important factors in the toxicity of iron salts and in the treatment of acute iron poisoning.

MATERIAL AND METHODS

Mature, female, albino mice of the Queckenbusch strain were used in all experiments. Unless otherwise stated, the mice were starved for 19 ± 2 hours before being given the test dose of iron, but at all times they had free access to water. During the period of starvation the mice were housed in a cage with a false bottom in order to prevent them eating sawdust or faeces.

The substance given, the route of administration and modifying factors are listed in Table I.

The intragastric doses were given through a fine polythene catheter with a heat-smoothed end, passed down the oesophagus while the mice were held immobilised by the skin at the back of the neck and by the tail.

All the solutions of iron salts were freshly prepared. The concentrations of the solutions were varied, so that the volume given to each animal (0.01 ml/g) was constant.

The slow intravenous doses were given through a 26 gauge needle, connected to a constant rate perfusion apparatus, inserted into one of the tail veins. The volume given was 0.01 ml/g over approximately 5 minutes. When rapid intravenous injection was used, the same volume was given in 5 seconds.

As controls, 20 mice were given intragastric distilled water (0.1 ml/g), and 5 were given intravenous isotonic saline over 5 seconds in each case with no ill effects.

When sodium bicarbonate was given orally or intraperitoneally, 0.1 ml. of sterile 9% sodium bicarbonate solution was given 10 minutes after the oral dose of ferrous sulphate.

When desferrioxamine (Desferal, Ciba) was given intramuscularly, 0.2 ml. of 10% solution was used. Diethylenetriaminepentacetate (DTPA, Geigy) was used as a 25% solution and again the volume used intramuscularly was 0.2 ml. The reason for these concentrations is that approximately the same amount of iron is chelated by equal volumes of 10% desferrioxamine and 25% DTPA.

An autopsy was performed on all animals that died within 4 hours, to ensure that accidental injection into the peritoneal or pleural cavity had not occurred. One drop of a 10% solution of desferrioxamine was placed on a swab from each of these cavities. The rapid development of the orange colour of ferrioxamine indicated the presence of iron solution, and when this colour change occurred, the affected mouse was excluded from the data and the experiment repeated.

The animals were maintained for at least one month after each experiment to allow for the development of possible late effects of poisoning, but the median lethal dose (LD_{50}) at 24 hours included virtually all the animals that died. The LD_{50} at 24 hours was obtained by using the tables of Weil (1952) when applicable. In other instances the graphic method of Litchfield *et al.* (1949) was used.

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1 Research Trainee

RESULTS AND DISCUSSION

The results are shown in Table I.

The $L.D._{50}$, or median lethal dose of intragastric ferrous sulphate to mice, has been determined by different workers. Somers (1947) gave a figure of 0.9 mg. Fe/gm., and Edge *et al.* (1948) estimated it to be 0.71 mg. Fe/gm. These animals had been starved for only 4 hours before the dose was administered. Hoppe *et al.* (1955) estimated the $L.D._{50}$ to be 0.306 mg. Fe/gm., also after 4 hours starvation. Weaver *et al.* (1961) did not mention whether their mice were fasting, and estimated the $L.D._{50}$ to be 0.226 mg. Fe/gm. Eickholt *et al.* (1965) fasted their mice for 22 ± 2 hours and obtained a figure of 0.32 mg. Fe/gm. They also demonstrated the marked difference in mortality depending on whether, during starvation, the mice had access to faeces, sawdust, or neither. When given a standard dose of ferrous sulphate solution (.048 mg. Fe/gm.), the mortality of mice housed in a cage containing faeces and sawdust was 35%. When the cage contained faeces without sawdust, the mortality rate was 55%, and when there was no access to faeces or sawdust the mortality rate was 95%. Eickholt *et al.* (1965) also demonstrated a slight sex difference in susceptibility to iron poisoning, and a moderate strain difference.

It can be seen from the above, and from the $L.D._{50}$ of ferrous sulphate solution estimated in the current series of experiments, 0.15 mg. Fe/gm. for starved mice and 0.36 mg. Fe/gm. for unstarved mice, that the range of figures is quite wide. Some of the factors which are important in producing these different results appear to be:

1. Differences in the susceptibility of different strains.
2. Length of starvation.
3. Access to faeces and/or sawdust during the period of starvation.

As well as these three points, Cambridge *et al.* (1966) demonstrated the influence of diet on the acute toxicity of injectable iron preparations, and the protection afforded by increased amounts of Vitamin E in the diet.

In order to obtain comparable results in the current series of experiments, all animals were starved for the same length of time, of the same strain, and none had access to sawdust or faeces during the period of fasting. Following weaning, content of Vitamin E in the diet was kept constant.

When ferrous sulphate is given intravenously, there is less variation in the reported median lethal dose.

Edge *et al.* (1948) estimated that the $L.D._{50}$ of ferrous sulphate solution given intravenously was 0.014 mg. Fe/gm, but no mention is made of the volume or rate of injection. Nissim (1953) reported a figure of 0.011 mg. Fe/gm, but again the rate and volume of injection are not mentioned. Hoppe *et al.* (1955), estimated the intravenous $L.D._{50}$ for mice to be 0.010 mg. Fe/gm when the solution was given in a volume of 0.01 ml/gm at the rate of 1.0 ml/min. Cambridge *et al.* (1966) obtained a figure of 0.022 mg. Fe/gm for mice fed on a diet supplemented with Vitamin E, and 0.007 mg. Fe/gm on a normal maintenance diet. The figure of .013 mg. Fe/gm obtained after rapid injection over 5 seconds in the current series of experiments agrees fairly well with other reported results, except those of Cambridge *et al.* When the same volume of solution was given over approximately 5 minutes, the $L.D._{50}$ was doubled to 0.027 mg. Fe/gm. The mode of death in these two groups appeared to be different. When the standard dose was administered by rapid intravenous injection, the respiratory rate increased, the mouse then had a convulsion followed by respiratory arrest, all of which occurred within one minute of the end of injection. If the animal survived, the tachypnoea slowly settled over the next 15 to 30 minutes, and at the end of this time the mouse appeared to be perfectly normal.

With slow intravenous injection, the mode of death appeared more akin to that following oral poisoning with ferrous sulphate, except that with the higher doses, the mouse frequently died during the course of the injection. The animal initially had a raised respiratory rate which slowly settled. The mouse then became depressed, making few spontaneous movements. The respiratory rate rose and respirations became shallow. Depression deepened into complete prostration which was followed by a terminal convulsion and respiratory arrest. Most of the deaths occurred within 6 hours. This

sequence is similar to that described by Hoppe *et al.* (1955), and to the results of intragastric injection in this investigation.

Somers (1947) compared the oral toxicity of a number of iron preparations and found that ferrous carbonate was much less toxic, presumably because of its insolubility, than the other compounds tested. He suggested that oral sodium carbonate, or sodium bicarbonate given intravenously, might be of value therapeutically, by converting the more soluble iron salts to insoluble carbonate. It has become fairly standard clinical practice to use a dilute solution of sodium bicarbonate for gastric lavage and to leave some in the stomach when the lavage is completed.

In our experiments it will be noted that when intragastric sodium bicarbonate solution was given 10 minutes after a dose of intragastric ferrous sulphate solution, the $L.D._{50}$ was 0.46 mg. Fe/gm. When the same amount of sodium bicarbonate was given intraperitoneally 10 minutes after intragastric ferrous sulphate, the $L.D._{50}$ was 0.45 mg. Fe/gm. The administration of the same amount of sodium bicarbonate, given intraperitoneally to 5 mice and intragastrically to 5 mice, produced no fatalities.

The use of a chelating agent is now a standard part of the management of acute iron poisoning. The two most popular agents are desferrioxamine (Desferal, Ciba) and diethylenetriaminepentacetate (DTPA, Geigy).

Desferrioxamine is the more specific iron-binding agent, but when given rapidly intravenously may produce hypotension (Whitten, 1965).

In the present series of experiments, desferrioxamine and DTPA were given intramuscularly at the same time as the ferrous sulphate solution was given intragastrically. Both chelating agents gave considerable protection from the iron.

CONCLUSIONS

By maintaining the experimental conditions constant, i.e. the factors which appear to influence oral toxicity, particularly those relating to acute iron poisoning, the results of different experiments can be compared. Because of the variability in these factors it is difficult to compare the figures obtained in different laboratories, as illustrated by Somers (1947) and Hoppe (1955).

Both estimated the $L.D._{50}$ for intragastric ferrous sulphate and ferrous gluconate. Somers' figures were 0.9 mg. Fe/gm and 1.1 mg. Fe/gm respectively. Hoppe estimated the $L.D._{50}$ to be 0.306 mg. Fe/gm for ferrous sulphate and 0.429 mg. Fe/gm for ferrous gluconate. While the actual figures differ greatly, the ratio of the $L.D._{50}$ of ferrous sulphate to ferrous gluconate is 0.82 (Somes) and 0.71 (Hoppe).

As one would expect, the $L.D._{50}$ of intravenous ferrous sulphate is considerably less than that of oral ferrous sulphate. The sudden death of the animal when the injection is given rapidly would suggest that factors other than iron toxicity, e.g. acute acidosis, may be involved. Death is unlikely to be due to acute circulatory overload, as the rapid injection of the same volume of saline to a control animal had no ill effect.

The usual explanation for the beneficial effect of sodium bicarbonate following acute iron poisoning is that the iron is made insoluble by the formation of ferrous carbonate and is thus not absorbed (Somers, 1947). This may not be the whole story for if the sodium bicarbonate is given *intraperitoneally*, the iron is less toxic. It is probable that in this latter case the bicarbonate combats the metabolic acidosis that is invariably present in animals poisoned by iron (Reissman *et al.*, 1955), and thus lowers the toxicity of the iron.

There is no doubt from the results we obtained that intramuscular desferrioxamine and DTPA afford considerable protection from acute iron poisoning.

SUMMARY

A series of experiments have been performed to determine some of the factors important in acute iron poisoning and its management.

The median lethal dose, $L.D._{50}$, has been determined for intravenous ferrous sulphate and ferric chloride and for intragastric ferrous sulphate modified by sodium bicarbonate, desferrioxide and DTPA.

Both intragastric and intraperitoneal sodium bicarbonate diminish the toxicity of intragastric ferrous sulphate.

Intramuscular desferrioxamine and DTPA were approximately equally effective in reduc-

TABLE I

Results of the Investigation

Iron Salt	Route of Admin.	Modifying Factor	Doses (mg.Fe/g) and Mortality								LD_{50} mg.Fe/g.	95% Confidence Level of LD_{50} mg.Fe/g.
1. Ferrous Sulphate	I.V. rapidly	—	.008	0/5	.010	0/5	.013	3/5	.017	5/5	.013	.012 — .015
2. Ferrous Sulphate	I.V. slowly	—	.027	2/5	.033	4/5	.040	4/5	.049	5/5	.028	.023 — .035
3. Ferrous Sulphate	I.P.	—	.030	0/5	.042	2/5	.060	4/5	.055	5/5	.047	.038 — .058
4. Ferrous Sulphate	I.G.	—	.060	2/10	.121	3/10	.242	8/10	.483	8/10	.15	.141 — .16
5. Ferrous Sulphate	I.G.	Unstarved	.121	4/10	.181	4/10	.272	6/10	.408	6/10	.36	.25 — .51
6. Ferrous Sulphate	I.G.	$NaHCO_3$ I.P.	.121	0/5	.242	1/5	.484	2/5	.968	5/5	.45	.29 — .70
7. Ferrous Sulphate	I.G.	$NaHCO_3$ I.G.	.199	2/10	.282	2/10	.399	3/10	.564	7/10	.46	.30 — .61
8. Ferrous Sulphate	I.G.	De feral	.399	1/5	.564	0/10	.798	4/5	1.128	4/5	.75	.55 — 1.01
9. Ferrous Sulphate	I.G.	D.T.P.A.	.399	0/10	.564	2/10	.798	2/10	1.128	8/10	.86	.72 — 1.03
10. Ferric Chloride	I.V. Slowly	—	.016	0/5	.028	0/5	.050	3/5	.091	5/5	.049	.037 — .065
11. Ferric Chloride	I.G.	—	.186	1/10	.335	2/10	.604	8/10	1.087	10/10	.44	.30 — .63

I.V. = intravenous
I.P. = intra peritoneal
I.G. = intra gastric
D.T.P.A. = Diethylenetriaminepentaacetate

ing the mortality following acute iron poisoning.

ACKNOWLEDGEMENTS

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**Surgical Research Unit,
Royal Children's Hospital,
Melbourne, Australia.**

FERROUS SULPHATE POISONING; REPORT OF A CASE

ARTHUR W. HOYT, M.D.
CHICKASHA, OKLAHOMA

INTRODUCTION

Most of us, in the past, have assumed that medicinal iron preparations are as innocuous as they are prevalent. However, fatal and non-fatal poisoning with Ferrous Sulfate has been the subject of several recent reports in the medical literature. Most of these reports have appeared in British medical journals and in pediatric journals in the United States. The following case is reported here in the hope that many physicians who do not regularly peruse either the pediatric or foreign literature may be made aware of the toxic potentialities of this widely used drug.

CASE REPORT

K. F., a white girl, age 19 months, was admitted to the Chickasha Hospital at 8:00 P.M., March 12, 1952, with complaints of being "drowsy" and "vomiting blood." She had been entirely well until about three hours prior to admission when she complained of her stomach hurting and became listless. One-half hour after onset of these complaints, she was offered her supper, refused most of it except a small amount of tomato soup, and immediately thereafter began to vomit large amounts of watery, red tinged material. Vomiting occurred approximately every 15 to 30 minutes up to time of admission to the hospital, and she became rapidly more drowsy. Her parents, alarmed by the first emesis, took her at once to their family doctor who referred her to the hospital for admission because of her obviously serious condition aptly described by him as "shocklike" and "collapse."

On examination the baby was noted to be well nourished and within normal limits of size for her age. She appeared to be critically ill: Temperature 96.8 (rectal), semicomatose, very pale, respiring normally, arousing only to vomit copious amounts of watery, pink tinged material with some strings of mucus and a few clumps of spongy, brown material. Her skin was cool and dry and there was no cyanosis. Increased peristaltic sounds were present all over the abdomen, which was not distended. Muscle tonus was generally decreased; tendon reflexes were decreased in amplitude and bilaterally equal. There was no remarkable odor on her breath. Blood pressure was not recorded.

While a catheterized urine and the vomitus were being checked laboratorywise, the neck, chest and abdomen were hastily viewed under fluoroscope, but no radio-opaque foreign body was seen. Under repeated questioning the father at this time recalled that his wife had missed some "big, brown, round, iron tablets" which she had been taking for anemia. Coincident with the father's recollection, the laboratory reported that the child's urine was normal while her vomitus which was negative for blood microscopically and chemically (Benzidine), was positive for iron (Prussian Blue Test.) A hurried call to the patient's home confirmed the absence of ten pills from a box labeled "Ferrous Sulfate" which was found where the child had dropped it.

Since it was now apparent that the patient was suffering from acute Ferrous Sulfate poisoning, her stomach was lavaged with tap water, until the return was clear and colorless. Despite her semi-comatose state she gagged readily, and it was felt that danger of aspiration was negligible. She was then placed in bed with external heat, and an I. V. infusion of five per cent glucose in water started. Three hours after admission (six hours after onset of symptoms) she passed a soft, tarry, black stool which was Benzidine negative, but positive for iron; no RBC's were present on microscopic examination. Shortly after this she became restless, cried occasionally, and her body temperature rose to 100.6 (rectal). Since neither vomitus nor stools contained blood, and since signs of shock disappeared about eight hours after onset, transfusion was not attempted.

The remainder of the patient's course was one of steady improvement. Relapse following initial improvement, as noted in some cases reported previously by others, did not

materialize. The baby vomited a small amount of clear fluid, one time, 16 hours after her admission lavage. She passed a second tarry stool 15 hours after admission. A repeat urinalysis on the second hospital day was normal. Blood count twelve hours after admission was normal except for hemoglobin of 8.4 gms. with RBC of 5.6 million and marked hypochromasia of RBC's on smear. It was felt that this was due to dietary inadequacy prior to her present illness. No other laboratory procedures were done. She was discharged apparently well, 36 hours after admission, and was reported by her family physician to be well one week later, after which she moved from the community and further contact with her was lost.

DISCUSSION

The symptoms, signs and course of this case are not remarkedly different from those previously reported by others except that the vomitus and stools disclosed no blood, either microscopically or chemically, despite their suggestive color. It is assumed that the initial color of the vomitus was derived from coloring used in the coating of the tablets. The manufacturer of the tablets could not be ascertained however, so this explanation remains a conjecture. A second and less likely explanation might be that the baby ate tomato soup shortly before onset of the vomiting. By hindsight, aided by reviewing the cases reported in the articles both here and abroad, it is obvious that this child might well have been given either blood or blood plasma in lieu of dextrose in water. Recovery was probably due to her inherent stamina and the fact that emeses and gastric lavage reduced the poison to a smaller dose than would be fatal for one of her age and weight. Nevertheless, her appearance and symptoms were most alarming.

It is worthy of emphasis that with the relative numerical decline in other causes of death of young children, accidental death, including poisoning, has assumed prominence among causes of death in this age group in recent years. Therefore, it follows that poisoning with Ferrous Sulfate should be more widely known to the medical profession, and steps taken to prevent its occurrence. As suggested recently in an editorial in the *Journal of the American Medical Association*,[10] the druggist should be encouraged to do his share by labeling precautions, and the prescribing physician should be careful to admonish his patients of the dire consequences which can follow ingestion of Ferrous Sulfate by children of the toddling and exploring age group.

SUMMARY

A non-fatal case of acute poisoning by Ferrous Sulfate in a 19 month old baby is reported. It is suggested that the possibility of poisoning by this widely used drug should be more generally known to physicians, and that physicians and druggists alike should take steps to prevent its occurrence.

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FERROUS SULPHATE WITH ASCORBIC ACID IN IRON-DEFICIENCY ANÆMIA

M. C. G. ISRAELS
M.D., M.Sc. Manc., F.R.C.P.
DIRECTOR AND CONSULTANT PHYSICIAN

A. V. SIMMONS
M.B., B.Sc. Manc., M.R.C.P.
SENIOR HOUSE-OFFICER

DEPARTMENT OF CLINICAL HÆMATOLOGY, MANCHESTER UNIVERSITY AND ROYAL INFIRMARY, MANCHESTER 13

Summary A tablet containing ferrous sulphate in slow-release form combined with ascorbic acid, has been used in patients with iron-deficiency anæmia. The daily rise in hæmoglobin over the first month of treatment was determined in forty-five patients. The preparation was marginally more effective than the same tablet without ascorbic acid, and was effective in some patients with iron malabsorption.

Introduction

IN an attempt to improve the absorption and reduce the unpleasant side-effects of oral iron, methods have lately been developed to provide slow release of iron salts mainly in the duodenum and jejunum where iron absorption is more efficient than in the stomach.

Ferrous sulphate in slow-release form ('Feospan', 'Ferrogradumet') has been used in this way. Ferrogradumet tablets, in which the drug is held in a plastic matrix with thousands of minute interstices, have been used in iron-deficiency anæmia (Webster 1962, Howard 1964, Israels and Cook 1965); Webster (1962) found the preparation effective and noted that side effects were no more frequent than with a placebo. The lack of side-effects is probably due to two factors: firstly, the release of iron from the interstices occurs only to a very slight extent in the stomach on contact with gastric juice (Heyman et al. 1962), and secondly, the dose of elemental iron in ferrogradumet (105 mg. per day) is slightly half that of standard iron preparations such as ferrous sulphate or gluconate. Israels and Cook (1965) found that the hæmoglobin rise per week in patients with iron-deficiency anæmia given ferrogradumet was equal to that obtained in such patients given double the amount of elemental iron per day as standard ferrous sulphate. The hæmoglobin rise they obtained was 0·672 g. per 100 ml. per week (0·096 g. per 100 ml. per day).

Ascorbic acid has been given as an adjunct to oral iron therapy for many years. The rationale behind its addition was that it was a potent reducing substance which should convert, in the alimentary tract, ferric to ferrous iron which was known to be more easily absorbed. The addition of ascorbic acid increased the absorption of food iron (Moore and Dubach 1955), and Brise and Hallberg (1962) showed that ascorbic acid increased ferrous-iron absorption from the gut providing the dose of ascorbic acid was sufficiently high. Little advantage was apparent unless 200 mg. ascorbic acid was given with every 30 mg. of ferrous iron; with this dosage ratio there was a definite increase in absorption while the increase was only small when 100 mg. ascorbic acid per 30 mg. ferrous iron was given.

'Ferrograd C' combines slow release ferrous sulphate in the form of ferrogradumet and ascorbic acid and contains ferrous sulphate 525 mg. (equivalent to 105 mg. ferrous iron) and ascorbic acid 500 mg. as sodium ascorbate; this combination gives a ratio of approximately 140–145 mg. ascorbic acid per 30 mg. iron, which comes midway between the ratios which caused slight and definite increase in iron absorption in Brise and Hallberg's (1962) experiments.

Patients and Methods

We have used ferrograd C in patients with iron-deficiency anæmia. Forty-five patients were given the preparation in a dosage of one tablet each morning before breakfast. Thirty-four patients had straightforward iron-deficiency anæmia which should have responded to treatment with any oral preparation; the diagnosis was determined by clinical features, blood-film appearances, and, in many cases, serum-iron determinations. Of the remainder, five had not responded to other oral iron preparations previously, five were found, on simple testing, to have iron malabsorption before treatment was started, and one was a 45-year-old woman with menorrhagia who was awaiting hysterectomy. Initial hæmoglobin concentrations were taken and the patients instructed about dosage. Hæmoglobin was estimated at weekly intervals for 1 month. From these figures the average daily rise in hæmoglobin in g. per 100 ml. was obtained for each patient.

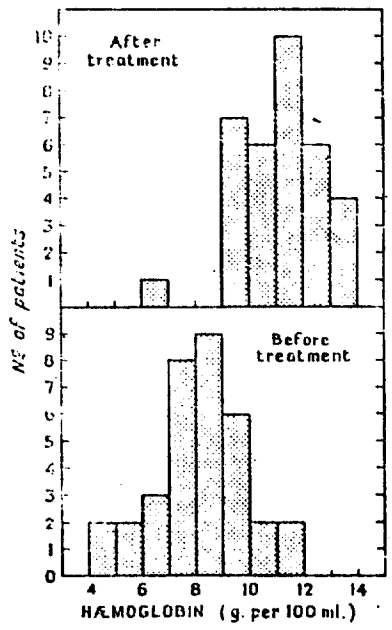

Fig. 1—Hæmoglobin, before and after treatment, in 34 patients with uncomplicated anæmia.

Results

Uncomplicated Anæmia

In these thirty-four patients, the average daily rise in hæmoglobin was 0·108 g. per 100 ml. (0·74%)

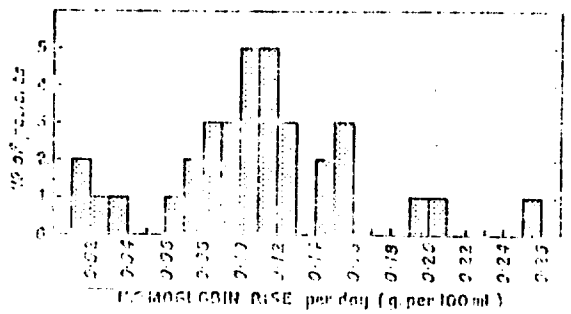

Fig. 2.—Haemoglobin rise per day, in 34 patients with uncomplicated anaemia.

per day (figs. 1 and 2). Four patients had very low mean daily increments of haemoglobin i.e., under 0·05 g. per 100 ml. per day.

Case 1.—Over a further 7 weeks on ferrograd C therapy, haemoglobin increased by a further 2·1 g. which increased her daily rise to only 0·031 g. per 100 ml., still under 0·04 g. per 100 ml. She had been previously investigated when her marrow was found to be normoblastic, her serum-iron 10 µg. per 100 ml., and her vitamin-B_{12} and folic-acid status normal. Her haemoglobin had then increased slowly but adequately on ferrogradumet. She had relapsed on stopping treatment and, later, ferrograd C had been given.

Case 2.—A man with rheumatoid arthritis who had a hypochromic anaemia with a low serum-iron. There was very little response to ferrograd C.

Case 3.—A man with proven pernicious anaemia who became anaemic whilst being treated with adequate dosage of cyanocobalamin. Bone-marrow showed micronormoblastic and megaloblastic hyperplasia.

Case 4.—This woman had previously responded well to ferrograd C and had relapsed on stopping treatment. Her serum-iron fell to 30 µg. per 100 ml.

The initial haemoglobin estimations (g. per 100 ml.) on these four patients were: case 1, 9·2; case 2, 7·6; case 3, 11·3; and case 4, 11·7.

If these four patients are excluded the average daily haemoglobin rise for the thirty patients is 0·120 g. per 100 ml. per day (0·032–0·307 per day).

Patients with other Deficiencies

Two patients with anaemia whose haemoglobin had not risen with [illegible] ferrograd C therapy increased their haemoglobin by 0·03 g. per 100 ml. Her initial haemoglobin was 8·4 g. per 100 ml. and her final haemoglobin 9·1 g. per 100 ml.

Malabsorption Tests

Malabsorption tests were carried out in these

[illegible]

Serum-iron rise (µg. per 100 ml.) after

Patient	Age	Sex	Ferrogradumet	Ferrograd C	Final	Daily rise
1	[illegible]	[illegible]	110	88	9·1	0·015
2	[illegible]	[illegible]	50	77	10·1	0·097
3	[illegible]	[illegible]	70	45	10·1	0·20
4	[illegible]	[illegible]	70	61	10·0	0·11
5	[illegible]	[illegible]	80	72	10·1	0·11

* Malabsorption confirmed by a rise in serum-iron of less than 100 µg. per 100 ml. 3 hours after ferromyn S alone, which was corrected in two patients by addition of 500 mg. ascorbic acid.

five patients. 10 ml. of blood was taken for serum-iron determination from the fasting patient who then took two tablets of 'Ferromyn S' (ferrous-succinate/succinic-acid mixture). Serum-iron levels were repeated 3 hours later. A rise of increase of 100 µg. per 100 ml. or more in the serum-iron indicates normal iron absorption. The iron preparation used in the test can be the preparation selected for treatment of the patient; but lately we have given all patients ferromyn S, since in our experience this has been the most effective oral iron tablet. We feel that iron malabsorption is certainly present if it occurs when ferromyn S is given. It is also better for comparison with other patients and for comparison with later tests in the same patient when a standard test is evolved. The test can be repeated with 500 mg. ascorbic acid taken orally at the same time as, or in addition to the iron. This test was performed in five patients of the seven patients and the malabsorption was corrected in two patients by the addition of ascorbic acid (table).

The average daily haemoglobin rise for this group was 0·11 g. per 100 ml. (0·7g %).

Only two patients out of forty-five spontaneously mentioned side-effects with ferrograd C. Patients were not directly asked about side-effects.

Discussion

The results in the patients in the first group can be used to assess the general usefulness of ferrograd C in the treatment of iron-deficiency anaemia. An average rise in haemoglobin of 0·11 g. per 100 ml. per day over the 3 months is comparable with the daily rise obtained under similar circumstances by Israëls and Cook (1965) with ferrogradumet and ferromyn S. The corresponding rises with these preparations were 0·095 and 0·143 g. per 100 ml. per day, respectively. The daily dose of elemental iron is similar in all three preparations. Ferrograd C is as effective as ferrogradumet and possibly slightly more effective but did not cause as high a daily haemoglobin increase as did ferromyn S. For such comparisons to be made it is important that the patients in different groups are similar. This is difficult in iron-deficiency anaemia since several pathogenetic factors may be at work. Dietary deficiency or unknown blood-loss, iron malabsorption may operate separately or together; in many cases there is no deficiency at all. Differing aetiological compositions of patients may be reflected in differing responses to treatment. The response also varies with the initial haemoglobin, folic-acid deficiency being a very potent enhancer of iron absorption. In the absence of malabsorption patients with low initial haemoglobin, say, below 7 g. per 100 ml., will usually do well whatever ferrous compound was given. Therefore the range of initial haemoglobin levels must be "similar" in series used for comparison. The patients described here were selected using the criteria of the Israëls ferrogradumet trial. Israëls and Cook had patients from the same clinic; indeed, an occasional patient had been in both trials having relapsed after stopping for various reasons to take ferrogradumet.

Ferrograd C tablets contain 500 mg. ascorbic acid and this provides a ratio of ascorbic acid to iron which previous experimental suggested would be sufficient to enhance iron absorption by any notable extent. Ideally, at least 700 mg. ascorbic acid should be included in the tablet. Size may be important here; the tablet as it stands is quite large, about twice the size of ferrogradumet, and the addition of 40% more ascorbic acid would increase its size to the point where swallowing might be difficult for some patients.

The patients in this series who had not responded to other oral iron preparations, did not respond well to ferrograd C. The number of patients in this category was small, and two of them were intolerant of ferrograd C and probably did not take it regularly.

Five other patients were found to have iron malabsorption after the test described above was done. The test is obviously not as informative as isotope studies of iron malabsorption but we have found it very useful clinically in deciding whether to treat patients with oral or parenteral iron. Patients unresponsive to oral iron, in the absence of intolerance of iron, nearly always have abnormal iron-absorption test results. Four of these five patients with iron malabsorption also underwent iron-absorption tests in which adequate amounts of ascorbic acid were added to the oral iron used in the test. This test corrected malabsorption in two cases. The response of all five patients to ferrograd C was almost equal to that found in patients without iron malabsorption despite the fact that the amount of ascorbic acid in ferrograd C is, theoretically, less than ideal. It would appear, therefore, that there is a place for treating patients with iron malabsorption with this preparation.

Ferrograd C tablets were provided by Abbott Laboratories, Kingsworth, Kent.

Requests for reprints should be addressed to M. C. G. I.

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A FATAL CASE OF FERROUS SULPHATE POISONING

BY

N. T. JACO, B.M., M.R.C.P.

and

R. C. B. PUGH, M.D.

(The Hospital for Sick Children, Great Ormond Street)

Iron salts have been used extensively in clinical practice for more than a century but the number of cases on record in which there have been severe toxic reactions is small. The earliest reports are those of Limouzin-Lamothe (1850), Chevallier (1850, 1858), Hall (1883) and Fitts (1888-9), who described five fatal cases of ferrous sulphate poisoning in adults. Since 1947, however, at least 25 cases of ferrous sulphate poisoning, 13 of them fatal, have been reported in the literature and it is significant that all but one of these patients were young children, usually between 1 and 2½ years of age (Forbes, 1947; Thomson, 1947 and 1950; Foucar *et al.*, 1948; Prain, 1949; Roxburgh, 1949; Smith *et al.*, 1950; Murphy *et al.*, 1951; Spencer, 1951; Duffy and Diehl, 1952; Smith, 1952; Swift *et al.*, 1952). There were 17 fatal cases in England and Wales in 1950 (B.M.J. 1953).

In recent years higher doses of ferrous sulphate have been used in treating hypochromic anaemia and attractive sugar-coated tablets resembling sweets are commonly dispensed. The tablets are used extensively in ante-natal clinics and expectant mothers are often given a month's supply (which may amount to 180 tablets) at one visit.

Toxic reactions are by no means confined to ferrous sulphate but have complicated the use of other preparations, such as proprietory products for intravenous use (Librach, 1953) and ferrous chloride (Lindquist, 1949). Moreover, experimental work in animals (Somers, 1947) has shown that a wide variety of ferric and ferrous salts are capable of producing necrotic changes in the gastric mucosa and degenerative changes in the liver when given in large doses. It is likely, too, that the frequency with which toxic reactions to iron salts occur is far in excess of the number of cases that are described in the literature, as it is probable that only the more dramatic examples are recorded. It is also well known that minor degrees of toxicity are relatively common and are frequently accepted as a personal idiosynerasy of the patient who is "unable to take iron", often because of gastro-intestinal discomfort or nausea.

This paper describes a further fatal case of ferrous sulphate poisoning in a young child.

CLINICAL FINDINGS

A.G., a 15-month-old male child, was left at home one morning with his elder brother, aged 3, while his mother went out shopping. The mother, who had been given an envelope containing 100 Ferrous Sulphate tablets the previous day at an antenatal clinic, left the house at 9.30 a.m. and returned half an hour later to find the envelope on the floor. The children had obviously been eating the tablets and at about 10 a.m. the patient vomited a quantity of dark brown material. Vomiting recurred three or four times during the following half hour and on each occasion similar dark brown vomit was produced. The child then had a convulsion and

became still and blue. The family doctor was called and, finding the patient unconscious, gave an injection of niketharnide and arranged for his immediate transfer to hospital.

On arrival at the Hospital for Sick Children at noon, the child was collapsed and cyanosed and was vomiting dark reddish brown material which had a peculiarly offensive metallic odour. The lips and nail beds were cyanosed; respiration was shallow and rapid; the pulse was weak, the capillary tone poor and the veins collapsed. The mouth and nasopharynx contained reddish-brown material that closely resembled the vomitus. Numerous coarse inspiratory and expiratory rhonchi were heard in all areas of the chest and it was presumed that he had inhaled some of the vomitus. The baby was comatose, but responded slightly to painful stimuli. There were no signs of meningeal irritation and no localizing signs in the central nervous system.

The vomitus, when tested with acid ferricyanide, gave an immediate strong positive Turnbull's blue reaction. A sample of blood mixed with water, as a control, gave a negative reaction with ferricyanide.

Immediate treatment was instituted; the stomach was aspirated and about 4 oz. (100 ml.) of dark brown fluid removed. The stomach was then washed out with 5 % Sodium bicarbonate and egg albumin. Oxygen was given and an intravenous drip of ¼ strength Hartman's solution and 2·5 % glucose was commenced, but death occurred at 2.15 p.m. approximately 4¼ hours after the ingestion of tablets.

A sample of blood taken immediately after death failed to clot even when left in a test tube for some hours. The serum iron content of this specimen was 20 mg. per 100 ml. (the normal range being 70-140 micrograms per 100 ml.).

Subsequent enquiry revealed that the child probably ingested 43 tablets of "Fersolate" (ferrous sulphate). The sibling was also examined at the hospital but, as he was symptomless and showed no abnormal signs on physical examination, was allowed to go home after his stomach had been washed out.

POST MORTEM FINDINGS

The post mortem was performed 19 hours after death. The body was that of a male child in good nutritional condition. Height 31½"; weight 25 lbs. (both within normal limits for his age). There was well marked cyanosis of the ears, lips and nail beds of the fingers and toes. The tongue was furred and the buccal mucous membrane was stained a light brown colour. A striking feature on internal examination was the complete absence of blood clot in the entire cardio-vascular system. The blood was still fluid at the completion of the autopsy.

Oesophagus: A small quantity of brownish mucoid material was lightly adherent to the epithelial surface, but there was no evidence of ulceration.

Stomach: Dilated and contained 15 ml. of opaque brownish, rather mucoid material, in which there was some altered blood and a few streaks of fresh blood. The wall was uniformly thickened and the mucous membrane showed numerous petechial haemorrhages and areas of superficial necrosis, while the rugae and the sulci between them were covered with a thick layer of dark brownish material, in which there was some mucus. The submucosa was oedematous and congested. These changes extended through the pylorus into the duodenum but did not extend in a proximal direction beyond the cardia (Fig. 1).

Small intestine: Contained about 35 ml. of reddish-brown mucoid material. The changes in the wall were similar to those seen in the stomach, but gradually diminished in severity, and were no longer evident when the upper ileum was reached. The lower ileum was congested.

FIG. 1. Stomach opened along the greater curvature, showing the thickening of the wall and discoloration of the mucous membrane. The lower end of the oesophagus is not discoloured and can be seen on the right of the photograph.

Large intestine: Contained some greyish-black material in its proximal portion. Mucosa normal throughout.

No tablets or portions of tablets were seen anywhere in the gastro-intestinal tract.

Trachea, main and branch bronchi: Filled with a large amount of the dark brown mucoid material, similar to that seen in the stomach (Fig. 2).

Lungs: Considerable oedema and congestion with lobular areas of collapse. On section frothy fluid exuded from the parenchyma and brownish mucus was seen in the bronchi.

Other organs: Brain congested and oedematous. Right heart dilated and the myocardium flabby and slightly paler than normal. Liver pale and flabby with an indistinct lobular pattern. Spleen twice average normal weight; Malpighian bodies large, pulp congested. Kidneys pale and flabby. Bladder contained 5 ml. of cloudy

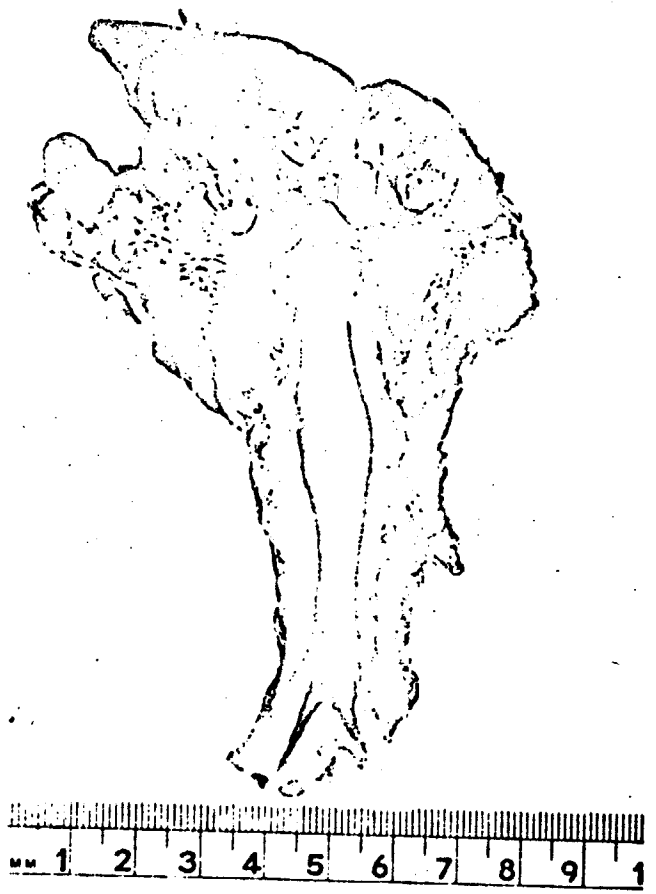

FIG. 2. Tongue, pharynx and trachea seen from behind. The lumen of the trachea is filled with inhaled vomitus.

urine. Tonsils large but not infected. Prominent upper cervical, mesenteric and hilar lymph nodes. Normal bone marrow in femur and sternum.

A macroscopic Prussian blue (acid ferrocyanide) test performed on small pieces of tissue excised from various viscera revealed the following results: stomach, duodenum, trachea, lung and liver—strong immediate positive; spleen and kidney—positive; sternal marrow and hilar lymph node—negative.

HISTOLOGICAL APPEARANCES

Sections were stained with haematoxylin and eosin, haematoxylin and van Gieson and Moore's elastic stains and by the Prussian blue (acid ferrocyanide) and Turnbull's blue (acid ferricyanide) methods, for ferric and ferrous iron respectively.

The lumen of the stomach contained masses of red cells, mucus and desquamated

epithelium. The mucous membrane was intensely congested, with many foci of recent haemorrhage; there was diffuse infiltration with lymphocytes and some polymorphs and numerous areas of superficial necrosis and ulceration were seen at the tips of the rugae. The submucosa was oedematous, contained several foci of recent haemorrhage and was diffusely infiltrated with chronic inflammatory cells. The mucosal and submucosal veins and capillaries were congested and frequently contained irregular masses of granular, often slightly basophilic, material which were interpreted as platelet thrombi. In haematoxylin and eosin preparations there was intense brownish discolouration of the superficial mucosa, the submucosal connective tissue and the walls of the mucosal and submucosal vessels (Fig. 3). Ferric iron was present in the gastric contents and a positive Prussian blue reaction was also given by the

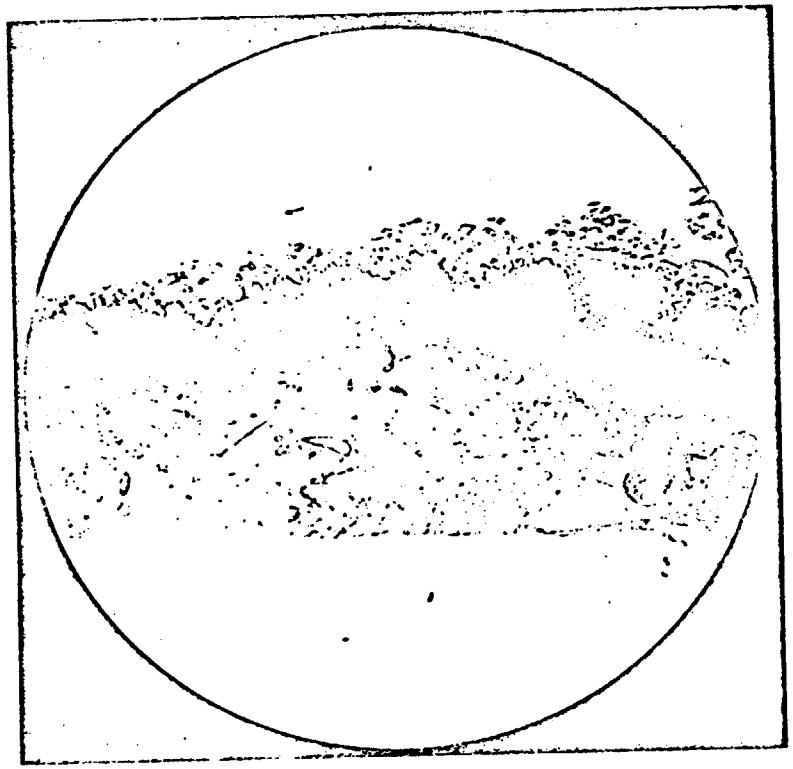

FIG. 3. Stomach. The lumen contains debris and there is superficial necrosis of the mucosa. The submucosa is oedematous and the vessels contain platelet thrombi. Haematoxylin and Eosin ×12.

reticulum of the superficial parts of the mucous membrane, by the submucosal connective tissue, the basement membrane and endothelial lining of capillaries, venules and lymphatics in the mucosa and submucosa. The platelet thrombi in the veins also stained deep blue and were seen either as a mass occluding the lumen or as a blue ring on the intimal surface of the vessel. The intima of several large subserosal veins contained iron, and granular iron was also present within the lumen of a few submucosal arterioles (Fig. 4). Sections stained with Turnbull's blue showed

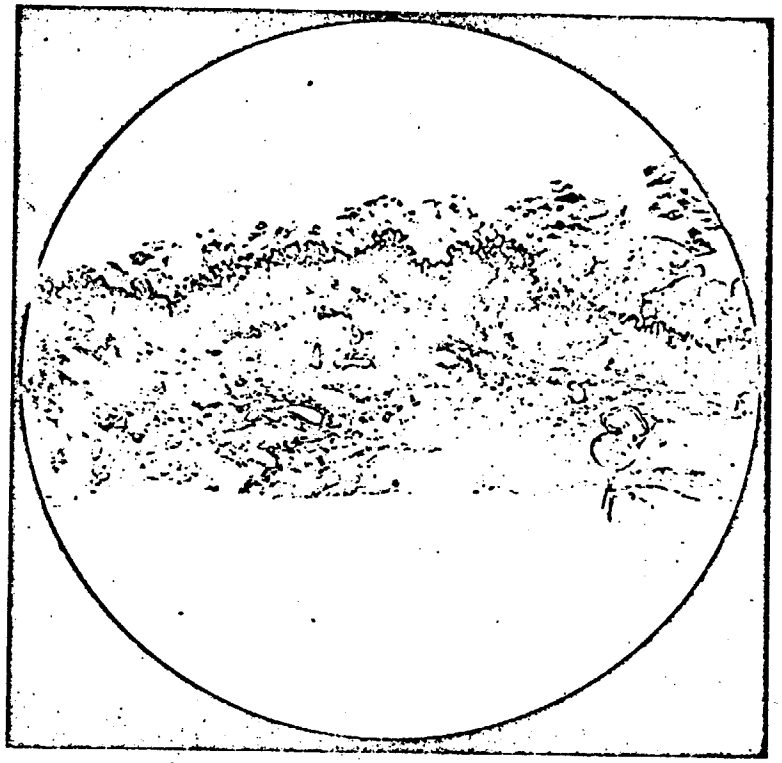

FIG. 4. Stomach. The same area as illustrated in Fig. 3, showing iron impregnation of the gastric contents, superficial mucosa, walls and contents of vessels and the submucosal connective tissue.
Prussian blue stain ×12.

an identical distribution of ferrous iron, although the intensity of the staining was less than by the Prussian blue method. In some areas the Turnbull reaction was more intense near the surface of the wall but elsewhere this distinction could not be made, so that there was a uniform blue colour at all depths from the mucosal surface. Similar changes were seen in the lower oesophagus, duodenum, jejunum and upper ileum, but there was progressive diminution in the severity of the lesions so that in the jejunum and upper ileum ulceration was not a conspicuous feature (Fig. 5).

The lower ileum and colon were not ulcerated but ferric and ferrous iron was seen on the surface of the mucous membrane, in platelet thrombi, in submucosal venules and, in granular form, within occasional subserosal arterioles. The lymphoid tissue in the bowel wall contained no iron, but ferric and ferrous iron was demonstrated in the littoral cells of the mesenteric lymph nodes.

In the liver there was fatty degeneration of the parenchymal cells at the periphery of the lobules, while the portal areas and several of the sinusoids contained lymphocytes, polymorphs and some eosinophils. Platelet thrombi were seen in many of the venous radicles in the portal tracts. Ferric iron was demonstrated in the connective tissue of the portal areas, in the endothelium of the portal veins, in the intravenous platelet thrombi, in the Kupffer cells and in the periportal reticulin framework of

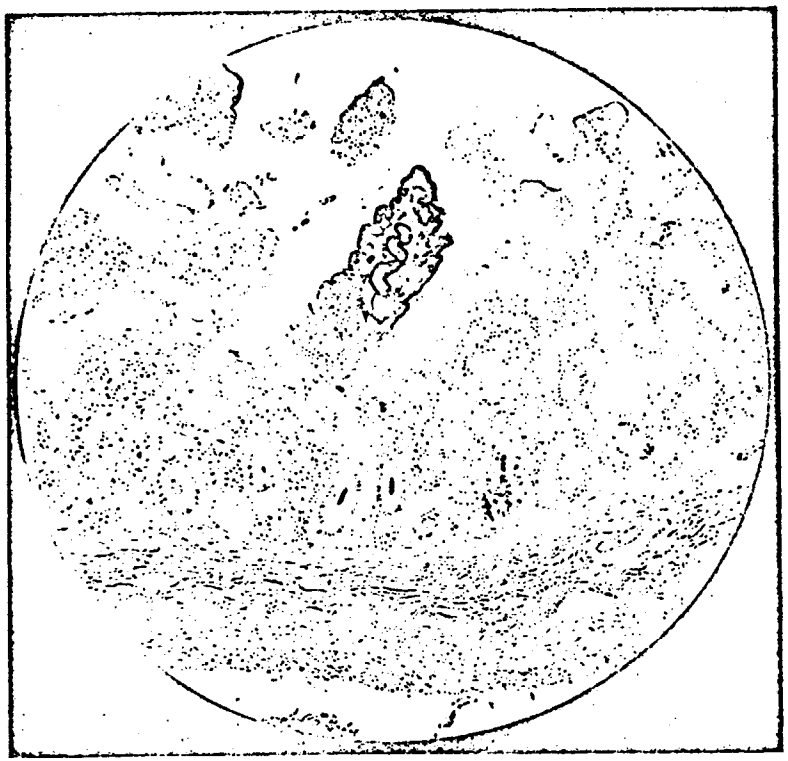

FIG. 5. Upper jejunum. In one area there is iron impregnation of the reticulum of the superficial mucosa. Many of the mucosal crypts contain material giving a positive reaction for ferric iron.
Prussian blue stain ×90.

the liver (Fig. 6). There was an identical distribution of ferrous iron, although the intensity of the staining was less than by the ferrocyanide method.

The trachea and some of the larger bronchi showed necrotic, inflammatory and vascular changes that were similar to those in the gastrointestinal tract. The lungs were congested and oedematous and contained lobular areas of collapse and numerous foci where gastric contents had been inhaled, with the accumulation of iron-containing debris within the alveoli and the production of an alveolar phagocyte or local polymorphonuclear reaction. Several small pulmonary artery radicles contained granular iron or small thrombi (giving positive reactions for ferric and ferrous iron).

Granular iron and iron-containing thrombi (giving a strong reaction for ferric iron and a weaker positive or negative reaction for ferrous iron) were seen in small arterioles and capillaries in the pituitary, brain and kidney. No iron could be demonstrated in the bone marrow or spleen. An occasional renal tubule contained eosinophilic debris, giving a positive reaction for ferric iron.

The brain was oedematous and several of the perivascular spaces in the parietal cortex contained compound granular corpuscles. There was considerable vascular congestion throughout the brain and many vessels in the white matter of the cortex

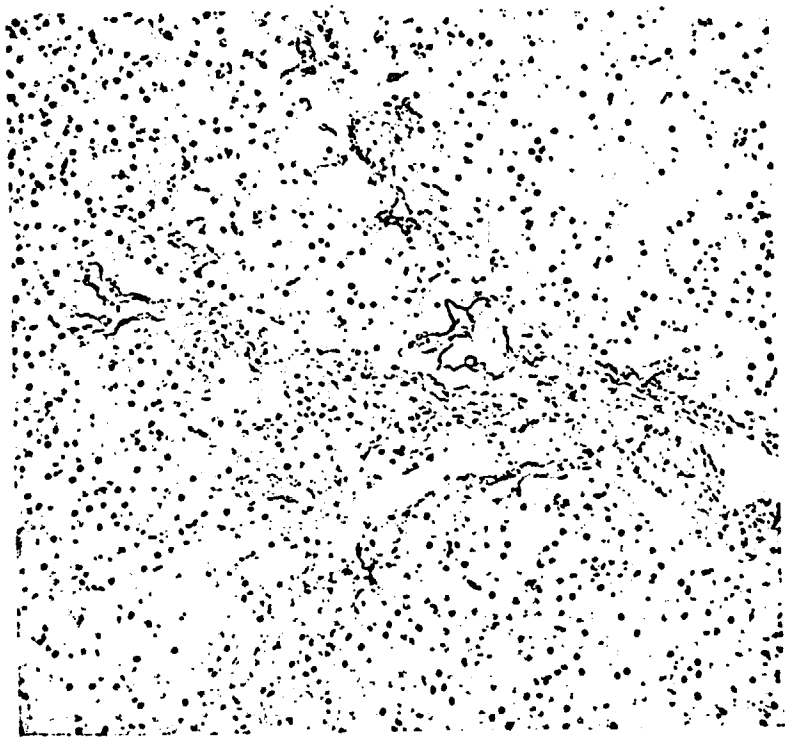

FIG. 6. Liver. There is impregnation of the periportal reticulum with ferric iron.
Prussian blue stain ×130.

and in the vicinity of the basal ganglia contained moderate numbers of polymorphs and lymphocytes, which were often marginated and arranged along the intimal surface of the vessels. Ischaemic cell change was seen in ganglion cells in the cornu ammonis and in some of the cranial nerve nuclei in the medulla. There was irregular loss of Purkinje cells in the cerebellar folia. There was a microscopic area of old softening, situated in the mid-line on the ventral surface of the corpus callosum immediately dorsal to the fornix, consisting of a central collection of compound granular corpuscles surrounded by a glial capsule.

Examination of the remaining viscera revealed no abnormality apart from oedema of the myocardium.

DISCUSSION

The clinical features of ferrous sulphate poisoning are well described in the literature and have been summarized by Spencer (1951), who outlined the possible sequence of events in young children, emphasizing that there are two critical phases in the illness. Death may occur between four and six hours after ingestion of the tablets and, if this initial danger period is survived, there may be a sudden and unexpected fatal collapse some 14 to 47 hours later. Within an hour of swallowing the tablets the patient looks pale and ill and frequently vomits. Initially the vomitus may contain some unaltered tablets and, in severe cases, it may contain small

quantities of bright red blood by about the end of the third hour. The child is now pale, cold, restless and drowsy, with a pronounced tachycardia: there is frequent retching and vomiting. Haematemesis may be frequent in the first 12 to 24 hours but diarrhoea is uncommon. Respiratory abnormalities, such as tachypnoea or irregular and shallow respirations, often occur. Spencer believed that death was due in the early stages to shock, as occurs with other corrosive poisons, and in the later stages to severe damage to the central nervous system. Forbes (1947) and Prain (1949) considered that death was due to liver damage, but Somers (1947) was unable to produce constant histological changes in the liver in experimental animals, and the lesions that he found were not sufficiently severe as to be likely to cause death.

The most recent and comprehensive account of the pathological lesions in a fatal case is that by Smith (1952), who confirmed the findings of previous observers and also described a number of new features. In seeking an explanation of the cause of death in the early critical phase, when the profound effects on the body often seem out of proportion to the histological changes seen at autopsy, he suggested that the shock-like state was due to an over-production of ferritin, which had been shown to possess marked vaso-depressor properties by Shorr, Zweifach and Furchgott (1945). The work of Hahn *et al.* (1943) and Granick (1946 a and b) has shown that when iron is ingested, ferritin (ferric hydroxide attached to a soluble specific protein "apoferritin") is formed in the mucosal cells and that, due to a selective action of these cells, iron absorption continues only until there is physiological saturation of the cells with ferritin. Smith (1952) postulated that a vast excess of iron in the stomach paralyses the selective absorption mechanism, with the result that an excess of iron is absorbed and converted into ferritin. The subsequent fate of ferritin is less well known but Granick (1943) has suggested that its iron content is released into the blood stream, where it combines with globulin.

Analysis of the commonly marketed brands of ferrous sulphate tablets shows that each tablet contains 3 grains of ferrous sulphate together with 1/25th grain each of copper and manganese sulphate. Although copper and manganese in large doses may produce toxic effects, Forbes (1947) demonstrated that the toxity of these tablets is due only to the ferrous sulphate they contain. In the majority of reported cases the emphasis has been on the effects of the iron component and the possibility that the acid radicles may produce a severe, and potentially lethal, acidosis has been largely ignored.

Many of the clinical accounts in the literature describe features, such as, for example, tachypnoea, which could be attributed to an acidosis. Further, in a recent case under the care of Dr. Reginald Lightwood, there was laboratory evidence of a severe acidosis. The patient was a child aged 15 months who retained 10 tablets of ferrous sulphate and who, prior to his complete clinical recovery, was found to have a plasma bicarbonate level of 29 vols. CO_2 per cent. Also, in the fatal case described by Swift, Cefalu and Rubell (1952) the CO_2 combining power of the plasma was 15 mEq. per litre a few hours after the ingestion of ferrous sulphate. Roxburgh (1949) reported the case of a child who swallowed 50 ferrous sulphate tablets (20 of which were subsequently vomited) followed by a quantity of Mist. magnesium trisilicate Co; the baby recovered completely in four or five days after intensive treatment with alkalies, penicillin and B.A.L., and it is tempting to attribute his recovery, in some part at least, to the initial ingestion of antacid.

The pathological lesions in this case are essentially similar to those hitherto reported and differ only in their extent. The presence of iron laden thrombi in the pulmonary and systemic circulation is probably related to the extremely high serum iron level (the highest so far recorded) and to the absorption of iron into the pulmonary circulation by diffusion from the inhaled vomitus. Smith (1952) failed to find such thrombi outside the portal venous system. The fluidity and lack of

spontaneous coagulability of the blood after death was noted but was not investigated in any way. It is not a feature that has been commented upon in any of the other fatal cases of ferrous sulphate poisoning but the investigations of Mole (1948) indicate that it can occur in a wide variety of conditions. It is less frequently seen in children than in adults and is most common when death is sudden and is preceded by shock or collapse, such as might occur in poisoning or drowning. An essential factor in its production is a shocked state and the appearance of the responsible fibrinolysin is thought to be part of the body's reaction to injury. The ischaemic cell changes in the cornu ammonis, cerebellum and cranial nerve nuclei are of recent origin and result from anoxia, almost certainly produced by the combined effects of the convulsions and the inhalation of vomitus. The area of softening just beneath the corpus callosum is clearly of some standing and cannot be related to the fatal illness, although its precise aetiology is not known. The immediate causes of death were anoxia and a profound shock-like state, presumably due to an excess of ferritin escaping into the circulation.

The possible sequelae in non-fatal cases have received little attention, but Spencer (1951) reported that abnormal liver function tests may persist for some time. Diarrhoea, though not a common feature of the early stages of the illness, may occur subsequently, with the passage of offensive stools containing iron and blood. (Spencer, 1951; Duffy and Diehl, 1952). Lindquist (1949) also noted a histamine refractory achylia, presumably due to damage to the gastric mucosa, in a two-year-old girl seven months after recovery from ferrous chloride poisoning. Crowskey (1952) and Ross (1953) reported two cases in which pyloric stenosis, due to scarring, developed two months after the ingestion of ferrous sulphate.

The necessity for taking more active steps to prevent these mishaps has been emphasized recently by one of H.M. Coroners, who records three inquests on fatal cases of ferrous sulphate poisoning in young children within the last few months and makes a plea for less indiscriminate use of inadequately controlled iron therapy for anaemia (Beecle, 1953). The potential dangers of the tablets have been stressed in many publications and by the manufacturers, but it is obviously of greater importance that the individual patients should clearly understand the hazards when the drug is prescribed. Then, with reasonable care in the home, these tragedies could be avoided.

In treating an established case, gastric aspiration and lavage should be performed immediately, and treatment of shock instituted. It is also suggested that biochemical investigations should be carried out as soon as the patient reaches hospital, and at frequent intervals thereafter, so that any tendency towards acidosis or other metabolic upset can be corrected immediately.

Summary

A fatal case of ferrous sulphate poisoning in a 15-month-old boy is described. Death occurred 4½ hours after the ingestion of 43 tablets and was due to anoxia from inhalation of gastric contents and to a profound shock-like state. The serum iron level was 20 mg. per 100 ml., which is the highest on record. The mechanism of production of shock and the cause of death in ferrous sulphate poisoning is discussed. Attention is drawn to the development of acidosis in some of these cases and certain broad principles of treatment are outlined. The possible sequelae in non-fatal cases are commented upon.

Our thanks are due to Dr. Reginald Lightwood for permission to publish this case and to quote a second case under his care; to Dr. Martin Bodian for the description of the gross autopsy findings and for his help in the preparation of the

manuscript: to Dr. W. W. Payne for the serum iron estimation; and to Mr. Derek Martin for the illustrations.

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FERROUS SULFATE POISONING

A Nonfatal Case

BENJAMIN B. KAPLAN, M.D.
AND
DONALD M. SCHLIEFER, M.D.
CHICAGO

FOR MANY years, iron, particularly the ferrous form, has been used as a medicinal agent in nutritional anemia and prepartal management. Since ferrous sulfate is relatively inexpensive, quite stable, not unpalatable, and, up to 1947, considered quite harmless, it has enjoyed wide distribution and in tablet form has been dispensed in large numbers with some abandon. This has resulted in easy availability and all too often accidental ingestion by the exploring and inquisitive infant or child.

Forbes,[1] in 1947, described postmortem findings in two cases. Thomson,[2] Prain,[3] Thomson,[4] and Smith[5] further documented the clinical and postmortem features of this condition. Spencer,[6] in 1951, reviewed the various problems involved and noted in Great Britain the beginning of branding procedures to the extent that "the drug is dangerous to young children." Duffy and Diehl[7] and Swift and collaborators[8] further established the clinical and postmortem picture. Veeder,[9] in 1952, reviewed 16 fatal and nonfatal cases and reemphasized the necessity for keeping iron preparations out of reach of infants and young children. Branch[10] reported a fatal case in a 29-month-old child, with death occurring four and one-half hours after the ingestion of ferrous sulfate tablets. Shoss,[11] early in 1954, reported a severe but nonfatal case of ferrous sulfate poisoning treated with dimercaprol (BAL).

REPORT OF CASE

J. M., a 13-month-old Negro girl, was first seen and admitted to the Cook County Hospital at about 6 p. m. on Nov. 11, 1953. The informant stated that approximately three hours prior to admission the infant was believed to have ingested between 20 and 30 tablets of a preparation that had been dispensed at a prenatal clinic. Identification was established as 3-grain (0.19 gm.) ferrous sulfate tablets. One-half hour to 45 minutes after ingestion, the infant began to retch and vomited some blood-stained material. Soon thereafter, she became increasingly drowsy and lethargic, and began to perspire freely; she was then brought to the hospital.

Physical examination disclosed a well-nourished Negro infant, obviously appearing acutely ill and mildly cyanotic, sweating, and in shock. Rectal temperature was 37.4 C. (99.3 F.); blood pressure, 76 systolic, 42 diastolic; respirations, 36 per minute, shallow, and labored; pulse, 156 per minute but strong. Dried crusts of blood were observed in both nares, on the tongue, and on the chin. The pupils were equal, somewhat constricted, and reacted to light. The neck was supple, but the patient responded very poorly to painful stimuli. A marked hypotonia and hyporeflexia were noted. Examination was otherwise negative, and the past history and family history were noncontributory.

The patient was promptly lavaged with copious amounts of sodium bicarbonate solution, and a considerable quantity of a thick viscid brownish red material was recovered, which contained one or two partially dissolved and fragmented tablets. Serum iron determination at this time was found to be 1.68 mg. per 100 cc. In our laboratories, the low normal range is 0.056 mg. and the high normal range, 0.168 mg. Other determinations at this time revealed NPN 26 mg., inorganic phosphorus 5.9 mg., icteric index 6, albumin 3.8 gm., globulin 2.2 gm., cholesterol 228 mg., alkaline phosphatase, 7.9 Bodansky units; cephalin flocculation 0, and thymol turbidity 4.1 units. No methemoglobin determinations were made. The patient was given 250 cc. of

From the Pediatric Service of Dr. I. Pat Bronstein.
From the Children's Division of the Cook County Hospital.

... blood and oxygen and maintained in a warm bed. After the transfusion, 500 cc. of 5% dextrose in water was administered intravenously. Penicillin and vitamin K therapy were started in appropriate dosages.

During the night, she had one large semiliquid tarry stool and an occasional small emesis of a greenish blood-stained viscid substance. The infant remained lethargic. On the following morning, there was considerable improvement. She was alert and active, with comfortable respirations and a strong regular pulse of 136 per minute. The rectal temperature was elevated to 38.7 C. (101.7 F.) but returned to normal within 24 hours and remained normal for the rest of the hospital confinement. Improvement was steady and continuous, and no evidence of a relapse was noted, as has been so frequently described in this type of poisoning. Serum iron determinations on the second, third, and fourth days of hospitalization were all within normal limits. Bone marrow studies, urinalysis, bleeding and clotting times, serologic reactions, Mantoux reaction (1:10,000), roentgenograms of the chest and long bones were essentially normal and noncontributory. A repeat liver profile on the seventh hospital day demonstrated no abnormalities. A hemogram 18 hours after admission showed hemoglobin, 80%; RBC, 4,670,000; WBC, 20,200, with a differential count of 40% polymorphonuclears, 20% bands, 1% eosinophiles, 30% lymphocytes, and 9% monocytes. The platelet count was not abnormal. Four days after admission, the hemogram revealed hemoglobin, 70%; RBC, 4,840,000; WBC, 11,050, with 12% polymorphonuclears, 1% bands, 2% eosinophiles, 75% lymphocytes, and 10% monocytes.

The infant was discharged eight days after admission, apparently completely recovered.

COMMENT

Since the report of Forbes,[1] in 1947, an increasing number of cases of ferrous sulfate poisoning have been documented in the literature. The clinical picture of vomiting, lethargy, shock, gastrointestinal hemorrhage, and high mortality has been amply emphasized. Early and aggressive therapy to combat the shock and collapse is essential. Relapses are to be anticipated, and when they occur, appropriate measures are to be taken.

A recommendation is advanced for adequate branding of the drug as a potential poison.

SUMMARY

A case of ferrous sulfate poisoning with recovery in a 13-month-old infant is reported. Laboratory data of serum iron determinations are presented to substantiate the diagnosis. Through December, 1952, at least 26 cases of ferrous sulfate poisoning have been documented.

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BRIEF COMMUNICATION

STERILIZATION OF MALE RHESUS MONKEYS BY IRON SALTS

AMIYA B. KAR, V. P. KAMBOJ AND AJIT GOSWAMI

Central Drug Research Institute, Lucknow, India

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Summary. A single local injection of ferrous sulphate or ferric chloride causes total destruction of the testis of adult rhesus monkeys. Histochemically, the injected iron is found to be localized in the tunica propria of the tubules and in the interstitium; it accumulates in the mitochondrial and the supernatant fractions almost in equal amounts. It seems that iron causes a generalized damage to the testis through properties common to other heavy metallic ions.

It has been reported that iron salts cause damage to the gonads of rats and guinea-pigs (Telkka, Kuusisto & Antila, 1956; Kamboj & Kar, 1964). The present report is concerned with the effect of these salts on the testis of rhesus monkeys.

Adult male rhesus monkeys (*Macaca mulatta*) of the Institute's primate colony (8·5 to 9·5 kg) were used in this study. The iron salts (ferrous sulphate and ferric chloride 0·08 m-mole/kg body weight/testis, in 3 ml sterile distilled water; a single injection) were injected directly into the testes under aseptic conditions. The control animal received sterile distilled water alone in a similar manner. For histochemical demonstration of iron (Fe^{++} and Fe^{+++}) paraffin sections of the testis fixed in 10% neutral formalin were processed according to the procedure given by Pearse (1954). The total cholesterol concentration of the testis was determined by a method employed in a previous study (Kar, Harishchandra & Das, 1963). For subcellular fractionation, the testis samples were homogenized in 0·15 M sucrose and the fractions were isolated by the procedure of Jones & Gutfruend (1961) using a Servall superspeed refrigerated centrifuge. Iron (as Fe^{+++}) was then estimated from the subcellular fractions by polarographic method using an American Optical 103000 Electropolarizer.

It will be evident from the results presented in Table 1 that 7 days after the administration of the salts only the absolute weight of the testis was reduced. However, after 210 days the testicular weight diminished both on absolute and relative basis. Macroscopically, the organ showed necrotic patches at 7 days but at the chronic stage it was only a small mass of yellow coloured tissue. The cholesterol concentration of the testis increased considerably at 7 days but declined relatively at 210 days. The percentage of water showed a substantial decrease only at the chronic stage (Table 1).

Histologically, the testis of the control monkey presented typical adult features. In the ferrous sulphate treated monkey (7 days) the tubular diameter was considerably reduced and the tunica propria was disintegrated. The seminiferous epithelium was reduced to eosinophilic debris. The Leydig cells were totally degenerated, the interstitial blood vessels were engorged; and fibrin had been deposited in the interstitium. Focal inflammatory changes were seen in the peripheral regions, and the tunica albuginea was thickened. In the corresponding (7 days) ferric chloride injected animal about 50% of the tubules were histologically similar to those of the ferrous sulphate treated monkeys. The diameter of the rest of the tubules was not appreciably reduced but the contour was peculiarly irregular with peritubular deposition of fibrous tissue.

TABLE 1

TESTIS WEIGHT OF THE RHESUS MONKEYS AFTER INJECTION OF FERROUS AND FERRIC SALTS

Monkey No. and status	*Testis wt*		*Cholesterol (mg/g)*	*Water (%)*	*Subcellular concentration of Fe^{+++} (µg/g)*	
	Absolute (g)	*Relative (mg/kg)*			*Mitochondrial fraction*	*Supernatant fraction*
K15—Control (distilled water)	23·9	2·0	0·21	87·4	2·8	0·04
K18—Ferrous sulphate (7 days)	13·9	2·3	5·51	80·3	—	—
K23—Ferric chloride (7 days)	16·1	2·4	5·34	79·0	—	—
K11—Ferrous sulphate (210 days)	1·15	0·21	0·74	44·9	5·83	5·1
K19—Ferric chloride (210 days)	4·05	0·70	1·41	52·2	5·5	3·6

The other features of the organ were comparable to those of the ferrous sulphate injected animal. At 210 days all the histological landmarks characteristic of the testis were absent. The organ consisted of cellular masses of phagocytic nature filled with a golden-yellow pigment. The spermatogonia, primary spermatocytes, spermatids and the Leydig cells of the control monkey testis showed strong positive reaction for sudanophilic lipids. The tubules of the ferrous sulphate and ferric chloride treated animals (7 days) showed a high concentration of lipid in the cellular debris but the interstitium was virtually negative. At 210 days the testes were found to be devoid of sudanophilic lipids.

Iron could not be demonstrated in the testis of the control monkey by the histochemical procedure; only the blood vessels and patches of tunica albuginea showed the characteristic blue staining for iron. In the ferrous sulphate and ferric chloride treated animals (7 days) strong positive reaction was seen in the tunica propria but the cellular debris was negative. In the interstitium the degenerated cellular elements and the engorged blood vessels stained intensely. At 210 days considerable amounts of iron (ferrous and ferric) were seen in the ground substance and the pigment-containing cells of the degenerated testis. In the control monkey the mitochondrial fraction of the testis contained more

iron (as Fe^{+++}) than the supernatant (Table 1). However, 210 days after injection of ferrous sulphate or ferric chloride the total iron increased substantially in both fractions. The concentration was slightly less in the supernatant fraction of the ferric chloride injected monkey.

Typical castration changes were evoked in the seminal vesicles and the prostate by the salts; the gonadotrophin content of the pituitary (assayed by the immature mouse uterine weight method) showed a consistent increase in the iron-treated animals.

The results of the present study show that a single local injection of ferrous or ferric salts causes an acute and irreversible degeneration of the monkey testis affecting equally the germinal and the endocrine portions. From the generalized nature of this damage it seems that the iron salts act on the testis through toxic properties common to other heavy metallic ions (Passow, Rothstein & Clarkson, 1961).

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THE EFFECT OF VARIOUS COLLOIDAL AND CRYSTALLOIDAL METALLIC COMPOUNDS IN NUTRITIONAL ANEMIA OF THE RAT*

H. L. Keil, Ph.D., and Victor E. Nelson, M.S., Ames, Iowa

Keil and Nelson[1] have recently made an extensive study of the rôle of copper and certain other elements and amino acids in the regeneration of hemoglobin. This work constituted an elaboration and verification of earlier data by Hart, Steenbock, and coworkers.[2] Goerner[3] states that salts of manganese, as well as of copper, are capable of increasing the hemoglobin of anemic animals when these salts are added to salts of iron. However, he observed that colloidal solutions of manganese and copper in the presence of colloidal solutions of iron have no hematopoietic effect. Myers and Beard[4] found that higher doses of zinc and magnesium retarded blood regeneration.

The experiments recorded in this paper were instituted in order to answer certain questions: First, does manganese act like copper in hematopoiesis? Second, are colloidal Fe and Cu utilized in hemoglobin building? Third, will Fe salts injected intraperitoneally cause regeneration in anemic animals? Fourth, what effect do Zn and Mg salts have on the development of anemia? Fifth, what are the minimum amounts of Fe and Cu required for regeneration of hemoglobin? Sixth, to what extent can different compounds of Cu be utilized in hematopoiesis?

EXPERIMENTAL

All of the experiments were performed on rats. The milk used for the production of anemia was collected directly into glass containers from pure bred Holstein cows, in order to avoid contamination with copper. The salts were examined spectrographically by a Hilger quartz prism spectrograph and shown to be pure. Carbon electrodes were used in the spectrographic examination. Hemoglobin content was determined by the Newcomer method. The animals were bled by the tail.

Table 1

Diet: Whole Milk Collected in Glass

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	14.6	100.0	16
2	10.6	72.6	16
4	6.2	42.4	16
6	5.0	34.2	15
8	3.8	26.0	13

It is evident from the data in Table 1 that the consumption of the whole milk used in these experiments produces a very marked anemia. Data have

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also been obtained which show that anemia results even though iron salts are added to this milk. On the other hand, animals receiving ordinary market milk plus iron salts develop anemia much more slowly. This is due to the fact that ordinary market milk contains more copper than milk collected directly into glass. The amount of copper in market milk is variable.

TABLE II

EFFECT OF IRON AND COPPER SALTS ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	3.7	100.0	12
2	9.6	259.3	12
4	13.0	351.0	12
6	15.2	410.0	12
8	14.9	402.5	12

Table II shows that copper is very potent in hematopoiesis. The animals were made anemic on whole milk, and when the hemoglobin had fallen to 3.7 gm. per 100 c.c., 0.50 mg. of Fe as $FeCl_3$ and 0.05 mg. Cu as $CuSO_4.5H_2O$ were added to the basal diet daily. When the rats were eight weeks of age the average hemoglobin was 402.5 per cent of that at the anemic level.

TABLE III

EFFECT OF IRON AND MANGANESE ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	6.8	100.0	10
2	6.6	97.0	10
4	5.2	76.5	8
6	4.8	70.6	6

Table III shows that the addition of 0.50 mg. of Fe as $FeCl_3$ and 0.10 mg. Mn as $MnSO_4.4H_2O$ daily to the milk failed to stimulate regeneration of hemoglobin. Manganese therefore cannot replace copper.

TABLE IV

EFFECT OF COLLOIDAL IRON AND COPPER SULPHATE ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	4.5	100.0	10
2	10.6	235.5	10
4	11.0	244.1	10
6	14.7	326.2	10
8	15.0	333.0	10

Table IV demonstrates that colloidal iron can be utilized in the building of hemoglobin. The colloidal iron was prepared from copper-free $FeCl_3$ solution. The latter solution was added to boiling Cu-free water, then dialyzed until free from dialyzable iron. After the animals had developed anemia, 0.50 mg. Fe as colloidal iron and 0.05 mg. of copper as $CuSO_4.5H_2O$ were added to the milk diet. The same results were obtained by the injection of the colloidal iron intraperitoneally.

TABLE V

EFFECT OF FERRIC CHLORIDE AND COLLOIDAL COPPER ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	4.6	100.0	10
2	10.0	217.3	10
4	13.5	293.5	10
6	15.2	330.4	10

The data in Table V show that colloidal copper is also utilized in hemoglobin formation. The copper was nondialyzable. The animals received milk until they were anemic, and then 0.50 mg. of Fe as $FeCl_3$ and 0.05 mg. Cu as colloidal copper were added.

TABLE VI

EFFECT OF COLLOIDAL IRON AND COLLOIDAL COPPER ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	4.0	100.0	12
2	7.8	195.0	12
4	10.6	265.0	12
6	12.0	300.0	12
8	12.9	322.5	12
10	13.8	345.0	12
12	14.6	365.0	12
14	15.1	377.5	12

Table VI shows that colloidal Fe and Cu are utilized for the construction of hemoglobin. The animals developed anemia, and then 0.50 mg. of Fe as colloidal Fe and 0.05 mg. of Cu as colloidal Cu were fed daily. The same results were obtained by injection of the iron and copper intraperitoneally.

TABLE VII

EFFECT OF INTRAPERITONEAL INJECTION OF IRON SALTS ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
	Ferric Citrate		
0	6.2	100.0	10
2	10.0	161.4	10
4	11.4	183.9	10
6	11.6	187.1	10
8	11.0	177.5	10
10	9.5	153.2	10
	Ferric Chloride		
0	6.8	100.0	18
2	9.6	141.2	18
4	10.2	150.0	18
6	11.0	161.7	17
8	11.2	164.6	17
10	10.7	157.2	16
12	9.2	135.3	16

Table VII shows that Fe as citrate when injected intraperitoneally results in a temporary stimulation of hemoglobin formation. This experiment was performed in order to ascertain if copper was concerned in the absorption of Fe. Copper may play some part in the absorption of iron, but the data show

clearly that the rôle of Cu in hemoglobin regeneration cannot be explained on this basis alone. $FeCl_3$ acts like iron citrate although it causes necrosis. $FeCl_3$ in glycerol, however, does not have this deleterious effect when injected. The Fe as citrate and chloride was administered at a level of 0.50 mg. of Fe daily.

TABLE VIII

EFFECT OF ZINC AND MAGNESIUM SALTS ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
	$ZnCl_2$ and $FeCl_3$		
0	12.5	100.0	6
2	11.0	87.9	6
4	9.4	75.2	6
5	8.7	69.6	6
	$MgCl_2$ and $FeCl_3$		
0	12.3	100.0	6
2	10.8	87.8	6
4	8.7	70.8	6
5	7.5	61.0	6
	$FeCl_3$		
0	13.1	100.0	6
2	11.4	87.0	6
4	9.7	74.1	6
5	8.8	67.2	6

The data in Table VIII show no effect of Zn and Mg on the development of anemia. The rate of fall of hemoglobin was the same with or without these elements. Two-tenths milligram of Zn as $ZnCl_2$ and 0.2 mg. of Mg as $MgCl_2$ were fed daily, together with 0.50 mg. Fe as $FeCl_3$. Myers and Beard[4] have

TABLE IX

EFFECT OF INTRAPERITONEAL INJECTION OF 0.002 MG. CU AS $CuSO_4.5H_2O$ DAILY

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	7.4	100.0	12
2	11.8	159.5	12
4	14.0	189.2	12
6	14.8	200.0	12
8	15.0	202.7	12

TABLE X

EFFECT OF INTRAPERITONEAL INJECTION OF 0.10 MG. FE AS $FeCl_3$ DAILY

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
0	6.0	100.0	12
2	8.9	148.3	12
4	10.4	173.3	12
6	11.8	196.6	12
8	13.2	220.0	12
10	14.3	238.3	12
12	15.1	251.6	12

stated that larger doses of Zn and Mg retard blood regeneration. We therefore believed anemia might develop more rapidly on milk plus $FeCl_3$ if these elements were included. The data show this is not the case.

Table IX shows that normal regeneration of hemoglobin is obtained by 0.002 mg. Cu as $CuSO_4.5H_2O$ injected intraperitoneally daily. The animals received milk *ad lib.* and orally 0.50 mg. of Fe as $FeCl_3$ daily. This is the smallest amount of Cu that would cause regeneration of hemoglobin to the normal level.

Table X illustrates that regeneration of hemoglobin to the normal level is obtained by injection intraperitoneally of 0.10 mg. Fe as colloidal ferric hydroxide together with 0.002 mg. Cu as $CuSO_4.5H_2O$. Smaller amounts of Fe failed to cause stimulation of hemoglobin formation to the normal level.

TABLE XI

EFFECT OF INSOLUBLE COPPER COMPOUNDS ON ANEMIA

TIME IN WEEKS	AVERAGE HB. (GM. PER 100 C.C.)	PER CENT OF ORIGINAL HB.	NUMBER OF ANIMALS
	Copper Sulphide 0.02 Mg. Cu		
0	6.0	100.0	6
2	11.0	183.3	6
4	12.3	205.0	6
6	11.5	191.6	6
8	11.7	195.0	6
10	12.0	200.0	6
12	13.4	223.3	6
14	14.8	246.6	6
	Copper Hydroxide 0.005 Mg. Cu		
0	6.1	100.0	6
2	11.2	183.5	6
4	12.0	196.7	6
6	12.8	210.0	6
8	14.1	231.2	6
10	13.8	226.3	6
12	14.2	232.8	6
14	15.0	246.0	6
	Cuprous Oxide 0.01 Mg. Cu		
0	4.2	100.0	6
2	8.8	209.5	6
4	11.9	283.5	6
6	13.6	323.8	6
8	14.6	347.0	6
9	14.8	352.0	6
	Cuprous Iodide 0.01 Mg. Cu		
0	5.0	100.0	6
2	9.4	188.0	6
4	11.3	226.0	6
6	13.5	270.0	6
8	14.5	290.0	6
9	15.1	302.0	6

The data in Table XI show that the animal body can utilize various copper compounds in the production of hemoglobin, even though these salts be very insoluble. The character of the anion apparently has no effect on the utilization of the copper.

SUMMARY

1. Manganese cannot replace Cu in the synthesis of hemoglobin.

2. Colloidal Fe and Cu are utilized in hematopoiesis.

3. Although the intraperitoneal injection of Fe salts into anemic animals causes a temporary rise in hemoglobin, it is evident that the main function of Cu is not in the absorption of iron.

4. Zinc and Mg have no effect in the development of anemia.

5. Two-thousandths (0.002) mg. Cu as $CuSO_4.5H_2O$ and 0.10 mg. Fe as colloidal $Fe(OH)_3$ are the minimum amounts of these elements that will cause a regeneration of the hemoglobin to a normal level in the anemic rat.

6. Cu_2O, CuS, $Cu(OH)_2$, and CuI are readily utilized by the anemic rat in hemoglobin building. The sulphide is less efficient than the other salts employed.

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KIMMEL*, C.A. and H.J. SCHUMACHER, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio. Interrelationships between nutrients and salicylate teratogenicity.

Continuing studies on the teratogenic action of salicylates are underway to determine the effects of nutritional deprivation or supplementation with concomitant salicylate treatment on the developing embryo. Wistar rats were maintained days 7 to 10 of gestation on a purified diet containing a marginally adequate amount of zinc to maintain normal pregnancy. Sodium salicylate, an effective teratogen as well as chelating agent, was given orally on day 9 of gestation in doses of 250 or 500 mg/kg and a significantly increased percentage of resorptions and malformations resulted. In another group of animals treated days 14 to 18 with sodium salicylate, offspring were found with missing or defective otoliths, an abnormality which Hurley (Fed. Proc. 27:193, 1968) reported to result from a manganese defitiency. It appears that salicylic acid may produce deleterious effects on the embryo by complexing with metals such as zinc or manganese to inhibit activity of metal-dependent enzymes essential in normal development. Other experiments were designed to supplement animals with metals capable of complexing with salicylic acid in an attempt to protect against the effects of this compound. However, supplementation with ferrous gluconate (2 mg) or manganous sulfate (3.2 mg) produced a striking increase in resorptions and malformations over those animals treated with salicylate alone. Control animals treated with ferrous gluconate or manganous sulfate produced normal offspring. Details of these results will be presented.

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Pyridoxine Deficiency and Iron Metabolism in the Pregnant Rat: Fetal responses [1,2]

AVANELLE KIRKSEY, JUDY A. DRISKELL AND ISOBEL E. MILLER
Foods and Nutrition Department, Purdue University, Lafayette, Indiana

ABSTRACT Iron intake of rats fed pyridoxine and pyridoxine-deficient rats was approximately doubled by oral administration of $FeSO_4$ supplements containing 2 mg elemental iron daily during gestation. Effects of the treatments on the iron content of fetal plasma and tissue storage fractions were investigated. Fetuses of deficient mothers had low levels of plasma iron and transferrin and increased transferrin saturation. These changes were associated with increased concentrations of total iron, non-heme and hemosiderin components in the fetus. The overall effect of the deficiency appeared to be an increase in iron transfer from placental to fetal tissues, with some mitigation by the low levels of transferrin in fetal plasma and of ferritin in placenta. The inability of iron supplements, administered to mothers fed the vitamin, to increase the iron content of fetal plasma or tissues indicated that iron passage from the placenta to the fetus was regulated. This control mechanism appeared to be operative, at least in part, in pyridoxine deficiency since iron supplements administered to deficient mothers whose tissues were replete with iron did not result in increased fetal iron content. The decrease in placental total ferritin content in deficient and in iron-supplemented mothers is discussed in relation to the regulation.

The possibility that pyridoxine is a factor in the regulation of iron absorption is controversial. The observations of Yeh et al.[3] and Neal and Pearson (1) have led to questions concerning earlier reports that pyridoxine-deficient rats (2) and swine (3) absorb large amounts of iron despite replete body stores. Recent data from this laboratory (4) suggested no major impairment in iron absorption in pyridoxine-deficient pregnant rats administered iron supplements orally during gestation, even though this is a period during which iron absorption is markedly increased (5, 6). Elevated levels of iron in liver and spleen of deficient animals were related largely to decreased utilization of iron in hematopoiesis and to reduced fetal demands. Alterations in iron storage components including increased hemosiderin-to-ferritin ratios, however, were observed in liver, spleen and duodenal tissues of deficient animals. The extent to which the deficiency may produce similar changes in placental tissue was of interest in view of Nylander's (7) suggestion that placental ferritin in the rat may play an important part in the transfer of iron from mother to fetus. Wöhler (8) and Bothwell et al. (9) have made similar postulations for maternal to fetal iron transfer in the rabbit. The present experiment was undertaken to determine in the rat whether pyridoxine deficiency per se, or in conjunction with oral iron supplements administered to the mother during gestation, leads to changes in placental and fetal iron components.

EXPERIMENTAL

Female rats of the Sprague-Dawley strain, 80 days of age, were used. Care of the animals and composition of the diets have been described previously (4). The basal diet contained 4 g Jones-Foster salt mixture (10) per 100 g, providing 0.2 mg elemental iron/g diet. Two groups of 10 animals each received pyridoxine-deficient diets for 3 weeks prior to mating and during the gestation period. Two additional groups received 8 μg pyridoxine/g diet for a comparable period of time. Mating was confirmed by the presence of sperm in a vaginal smear. Throughout the gestation period one deficient group and one group fed pyridoxine were given daily, by stomach tube, 1 ml of a solution of

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[2] Supported in part by The Nutrition Foundation, Inc.
[3] Yeh, S. O. J., B. Padella and B. F. Chow 1962 Iron absorption by vitamin B_6-deficient rats. Federation Proc., 21: 468 (abstract).

$FeSO_4 \cdot 7H_2O$ in 1% HCl, equivalent to 2 mg elemental iron.

On day 21 of gestation, animals were anesthetized by chloroform and the uterus removed intact. The uterine wall was split and the intact placenta and sac of each fetus was removed. Amniotic fluid was withdrawn by micropipette from each sac and pooled. Fetuses were removed and implantation sites, number and weights of live young were recorded. The placenta was separated from the sac and umbilical cord, blotted and weighed.

Blood was withdrawn from each fetus by cardiac puncture. The technique described by Grazer (11) was modified to eliminate a transfer of blood from the syringe by the insertion of a heparinized capillary tube in the system between the needle and syringe.

Hemoglobin was determined as oxyhemoglobin using 0.02 ml blood and measuring the absorbance at 545 mμ.

Plasma and amniotic fluid iron concentrations were determined by an ultramicro adaptation of the method of Trinder (12) using 20-μl samples. Total iron binding capacity of plasma and amniotic fluid was determined according to procedures reported previously for maternal plasma (4). Percentage saturation of iron binding capacity was obtained by dividing plasma iron concentration by total iron binding capacity.

Fifty-microliter samples of amniotic fluid were analyzed for total protein according to a modification of the method of Kingsley (13).

Placental and fetal tissue samples were dried at 110° and wet-washed according to the method of Reitz et al. (14). Total iron content was determined spectrophotometrically by the method of Sandell (15) using ortho-phenanthroline reagent.

Placental and fetal tissue samples were homogenized, diluted with deionized–distilled water and centrifuged at 2000 × *g*. Water-soluble ferritin and water-insoluble hemosiderin were separated by the procedures described by Kaldor (16), and the iron content of each fraction was determined by the method of Sandell (15).

The data were analyzed by analysis of variance techniques (17).

RESULTS AND DISCUSSION

Average numbers and weights of live young were presented in a previous report (4). The vitamin deficiency resulted in significant increases in fetal resorptions, decreases in both numbers and weights of live young and high placental-fetal ratios. Although the fetus is believed to draw pyridoxine from maternal blood (18) the observations of this study indicated that inadequate amounts of the vitamin were withdrawn from deficient mothers to provide for normal fetal growth. Iron supplementation per se did not significantly affect reproductive performance in deficient animals or in animals fed the vitamin.

Levels of hemoglobin in maternal or fetal blood were not influenced by pyridoxine deficiency (table 1). This is in contrast to the adverse effects of the deficiency on fetal growth and suggests priorities in the utilization of the vitamin. Pyridoxine deficiency in the mother resulted in polycythemia, low mean corpuscular hemoglobin (MCH) and low mean corpuscular volume (MCV) without changes in hemoglobin concentration (4). It is possible that hematological measures not assessed in the fetus were also altered by the deficiency. Hemoglobin levels of maternal blood in all groups were consistently higher than those of fetal blood. Iron supplementation did not change the levels in maternal or fetal blood in any group. Furthermore, the supplement did not prevent the decrease in hemoglobin concentration sometimes attributed to hemodilution of pregnancy (4). Values for pregnant animals, regardless of the level of mineral fed, were significantly less than for nonpregnant animals fed the same diet.

Concentrations of iron, total iron binding capacity (transferrin) and percentage saturation of iron binding capacity (transferrin saturation) of maternal and fetal plasma and amniotic fluid are presented in table 1. A concentration gradient between maternal and fetal plasma iron levels was evident for all groups with fetal values exceeding maternal values. Values for both fetal and maternal plasma exceeded those for amniotic fluid. The transferrin saturation data in this study sug-

TABLE 1

Concentrations of maternal and fetal hemoglobin and of iron, total iron binding capacity (transferrin) and percentage of saturation of iron binding capacity (transferrin saturation) in maternal and fetal plasma and amniotic fluid

	Pyridoxine fed Fe supplemented		Pyridoxine deficient Fe supplemented		Treatment significant ($P < 0.01$)
	0 mg	2 mg	0 mg	2 mg	
Hemoglobin, g/100 ml					
Maternal	14.7 [1] ± 0.6	14.8 ± 0.9	14.2 ± 0.7	14.6 ± 0.9	
Fetal	11.2 ± 0.3	11.0 ± 0.5	11.0 ± 0.4	11.3 ± 0.5	
Iron, μg/100 ml					
Maternal plasma	88 ± 4	105 ± 5	135 ± 4	149 ± 3	Vitamin
Fetal plasma	250 ± 5	233 ± 3	207 ± 4	215 ± 5	Vitamin
Amniotic fluid	60 ± 10	62 ± 7	45 ± 8	47 ± 9	Vitamin
Transferrin, μg/100 ml					
Maternal plasma	319 ± 10	329 ± 8	345 ± 9	352 ± 11	Vitamin
Fetal plasma	374 ± 12	324 ± 10	277 ± 4	293 ± 5	Vitamin
Amniotic fluid	130 ± 12	133 ± 10	100 ± 4	98 ± 5	Vitamin
Transferrin saturation, % [2]					
Maternal plasma	27.6 ± 1.2	31.9 ± 1.5	39.0 ± 1.1	42.3 ± 1.0	Vitamin, Fe
Fetal plasma	66.8 ± 2.0	71.9 ± 1.8	74.7 ± 1.2	73.4 ± 1.4	Vitamin
Amniotic fluid	46.1 ± 6.8	46.6 ± 6.2	45.0 ± 8.2	47.9 ± 9.0	

[1] Averages for 10 rats ± SEM.
[2] Iron (micrograms per 100 ml)/transferrin (micrograms per 100 ml), multiplied by 100.

gest a passive exchange across a semipermeable membrane could be via the pathway fetal to amniotic to maternal. A clear gradient between maternal and fetal transferrin saturation was apparent, thus emphasizing the active transport role of the placenta in exchanges of iron between the maternal and fetal systems. Similar relationships were observed in all groups including those deprived of vitamin and those receiving iron supplements.

The elevated levels of plasma iron and transferrin in deficient mothers may be related, in part, to less expansion of blood volume in the deficiency (19). In support of this suggestion the concentrations of these constituents in control and deficient nonpregnant groups were not significantly different (4). The possibility of increased absorption, however, is not ruled out. Increased transferrin saturation was observed in both maternal and fetal plasma of deficient groups, and in maternal plasma of iron-supplemented groups. These marked increases in transferrin saturation in maternal circulation are consistent with increased iron absorption. The increased saturation of maternal blood could conceivably lead to increased placental iron transfer to the fetus. The data on fetal iron concentration (table 2) support, in part, this suggestion since tissue concentrations were elevated in deficient groups. Further elevation, however, was not evident in fetuses of deficient mothers receiving iron supplements.

The low concentrations of iron and transferrin in plasma of fetuses of deficient mothers were paralleled by decreases in those values in amniotic fluid. Changes in transferrin levels are generally believed to be unrelated to iron absorption but the mobilization and transport of iron are protein dependent. Increases in transferrin and serum iron have been correlated with decreases in liver iron (20). Morgan (21) suggested that the level of transferrin reflected changes in its rate of cellular synthesis or destruction, or its withdrawal or destruction by the placenta. It is possible that pyridoxine, which is a necessary cofactor in the metabolism of many amino acids, was not adequate in the fetus for the synthesis of the protein moiety which binds iron.

Iron supplementation did not alter any of the parameters of plasma iron that were assessed in either maternal or fetal blood

TABLE 2

Concentration of iron and iron storage components in placenta and fetus, and of iron in maternal liver, spleen and duodenum

	Pyridoxine fed Fe supplemented		Pyridoxine deficient Fe supplemented		Treatment significant ($P < 0.01$)
	0 mg	2 mg	0 mg	2 mg	
Placenta					
Weight, g	0.502 [1] ± 0.038	0.482 ± 0.031	0.424 ± 0.070	0.434 ± 0.088	Vitamin
Total iron, μg	31.6 ± 2.0	26.8 ± 2.5	22.4 ± 1.8	23.0 ± 1.5	Vitamin
Total iron, μg/g wet tissue	63.3 ± 3.1	56.2 ± 2.1	53.2 ± 4.1	53.4 ± 4.0	
Heme iron, μg	23.0 ± 1.5	19.7 ± 1.3	13.1 ± 0.9	15.3 ± 1.0	Vitamin
Heme iron, μg/g wet tissue	46.2 ± 3.0	41.3 ± 3.2	31.2 ± 2.5	35.7 ± 3.0	Vitamin
Non-heme iron, μg	8.5 ± 0.8	7.1 ± 0.4	9.3 ± 0.7	7.7 ± 0.5	Fe
Non-heme iron, μg/g wet tissue	17.1 ± 1.5	14.9 ± 1.2	22.0 ± 2.0	17.7 ± 1.6	Vitamin, Fe
Ferritin, μg	6.5 ± 0.5	5.3 ± 0.4	5.5 ± 0.4	4.6 ± 0.3	Vitamin, Fe
Ferritin, μg/g wet tissue	13.0 ± 1.0	11.1 ± 1.1	13.0 ± 1.1	10.6 ± 1.0	Fe
Hemosiderin, μg	2.0 ± 0.1	1.8 ± 0.1	3.8 ± 0.2	3.1 ± 0.2	Vitamin
Hemosiderin, μg/g wet tissue	4.1 ± 0.3	3.8 ± 0.2	9.0 ± 0.4	7.0 ± 0.5	Vitamin
Hemosiderin/ferritin ratio	0.316 ± 0.012	0.342 ± 0.018	0.692 ± 0.022	0.670 ± 0.023	Vitamin
Fetus					
Weight, g	4.5 ± 0.4	4.4 ± 0.7	3.3 ± 0.7	3.2 ± 0.7	Vitamin
Total iron, μg	166.4 ± 10.1	154.5 ± 12.4	165.5 ± 16.0	157.5 ± 15.0	
Total iron, μg/g wet tissue	37.2 ± 3.2	35.6 ± 3.1	50.1 ± 5.0	49.1 ± 4.2	Vitamin
Heme iron, μg	22.3 ± 2.1	22.4 ± 2.0	17.3 ± 1.4	22.1 ± 2.0	
Heme iron, μg/g wet tissue	5.2 ± 0.3	5.2 ± 0.2	5.0 ± 0.3	7.1 ± 0.4	
Non-heme iron, μg	144.1 ± 14.0	132.1 ± 10.1	148.3 ± 7.1	135.4 ± 8.0	
Non-heme iron, μg/g wet tissue	32.0 ± 2.8	30.4 ± 1.9	45.1 ± 2.5	42.0 ± 3.0	Vitamin
Ferritin, μg	108.1 ± 7.1	101.0 ± 8.0	89.2 ± 6.1	87.2 ± 6.0	Vitamin
Ferritin, μg/g wet tissue	24.0 ± 1.8	23.1 ± 2.0	27.1 ± 2.1	27.0 ± 2.2	
Hemosiderin, μg	36.0 ± 2.5	31.1 ± 3.0	59.1 ± 4.0	48.2 ± 3.2	Vitamin
Hemosiderin, μg/g wet tissue	8.0 ± 0.5	7.3 ± 0.4	18.0 ± 1.2	15.0 ± 1.0	Vitamin
Hemosiderin/ferritin ratio	0.333 ± 0.014	0.304 ± 0.010	0.663 ± 0.020	0.552 ± 0.022	Vitamin
Maternal tissues					
Liver, total iron, μg	2112 ± 144	2946 ± 162	2665 ± 158	3276 ± 143	Vitamin, Fe
Spleen, total iron, μg	843 ± 92	955 ± 81	1117 ± 113	1151 ± 169	Vitamin
Duodenum, total iron, μg	19.7 ± 3.6	36.0 ± 4.1	41.7 ± 4.3	60.6 ± 4.8	Vitamin, Fe

[1] Averages for 10 rats ± SEM.

of any group. Iron supplements did not, therefore, prevent the drop in serum iron concentration observed in fetuses of deficient mothers. Perhaps the transport of iron in the deficiency had reached a maximum as indicated by the high percentage of transferrin saturation. In addition, the low transferrin levels observed in the deficiency possibly increased the difficulty of transporting the supplemental iron from the placenta to the fetal circulation. During the 3-week experimental period 42 mg elemental iron were administered orally to the mothers in addition to a comparable level fed in the diet. Wöhler (8) found that 30 mg iron sorbitol or saccharate injected into mothers resulted in only slight increases in serum iron, and that chronic medication with three 30-mg doses for 2 weeks did not lead to inundation of the fetus.

The concentrations of iron storage components in placenta are shown in table 2. Total iron per placenta was less for deficient animals than for animals fed the vitamin, but the concentration of iron per gram of tissue was similar. The concentration of heme and total heme was less and concentration of non-heme iron was slightly elevated in placentas of deficient mothers compared with groups fed the vitamin. The former is in contrast to the observation that the concentration of hemoglobin in maternal or fetal blood was not influenced by the deficiency. It is well established, however, that pyridoxal phosphate participates in the formation of an activated glycine derivative and also acts as a coenzyme in decarboxylation of α-amino-β-ketoadipic acid in the pathway of porphyrin synthesis.

Concentration of ferritin in the placenta was not influenced by the deficiency, but hemosiderin was increased resulting in an increased hemosiderin/ferritin (H/F) ratio. Although the concentration of ferritin was not affected by the deficiency, the quantity per placenta was less. If placental ferritin, or hemosiderin, or both, are involved in the transport of iron from the mother to the fetus as suggested by Wöhler (8) and Bothwell et al. (9), the total amount of ferritin may be of more importance than the concentration. If this assumption is true, then the decrease in ferritin could be a partial explanation for the decreased iron levels in fetal plasma and amniotic fluid, and the failure to transport large excesses of iron following iron supplementation of deficient mothers.

Neither total iron content nor concentration in placental tissue was affected when iron intake was about doubled by oral iron supplements administered during gestation. Wöhler (8) observed that even in the presence of maximal maternal serum iron values and elevated iron in all organs of the mother following short-term and long-term administration of large doses of injectable iron to rabbits, only slight increases occurred in the total iron content of the placenta. Morgan (21) found no difference in placental iron content in rats that were iron loaded or depleted prior to gestation. The decrease in nonheme iron in placenta of supplemented animals in this study is associated primarily with a decrease in ferritin. The decrease in this fraction may be related to the control of iron transport to the fetus.

Concentrations of iron and iron storage components in the fetus are shown in table 2. Total iron content per fetus was not affected by the vitamin deficiency but concentration per gram of tissue was markedly increased. The high concentration of iron in fetal tissues in the deficiency and the low concentration in fetal plasma are consistent with the increased transferrin saturation and the low transferrin levels, respectively, observed in the fetus. Increased transferrin saturation has been associated with increased iron transport from the placenta to the fetus. Iron transport, however, was not further increased by doubling the iron intake of the mother during gestation. Iron supplementation of mothers fed the vitamin did not increase fetal iron content or affect the storage forms.

In pyridoxine deficiency the pattern of change in fetal iron components was similar to that found in placental tissue. Total ferritin was less in the deficiency and hemosiderin was elevated resulting in a significantly higher H/F ratio. The significance of these changes is not known. Both

iron fractions are normally used in physiological functions and the total level of hemosiderin did not appear to be excessive enough to be considered toxic. No significant difference in total non-heme iron content in fetuses of deficient mothers was observed but concentration of non-heme iron was higher. This may serve some advantage in low birth weight young of deficient mothers since non-heme iron is a storage form which can be used for the formation of essential heme compounds during early life when rapid growth increases the need for these compounds. Pyridoxine is known to be essential for heme production and may have been used preferentially for this function because fetal growth was depressed by the deficiency without any alteration in heme concentration or total quantity.

Total iron content in maternal liver, spleen and duodenum are shown in table 2. Neither the deficiency nor the level of iron supplied to the mother influenced total fetal iron, whereas iron was considerably elevated in maternal liver and duodenum by both the deficiency and iron supplementation. Similar findings also have been observed in response to injectable iron compounds in the rabbit (8). Chronic medication of the mother failed to lead to inundation of the fetus although liver, kidney, heart, lung, muscle and spleen values were considerably increased in the mother. The investigator demonstrated by histochemical techniques that the administered iron was in the maternal vessels and the intervillous spaces but the descending fetal vessels were almost devoid of iron. The observations of the present study also indicated that when maternal tissues were replete with iron following iron supplementation the passage of iron from the placenta to the fetus was not increased. Furthermore, the control of absorption was operative, at least partially, in pyridoxine deficiency. Iron supplementation in the deficiency did not result in increased fetal iron. The decreased ferritin observed in placentas of both vitamin-deprived and iron-supplemented mothers may have served some regulatory function in preventing an excessive transfer of iron to the fetus.

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The Intestinal Absorption of Ferric Ion Administered Orally

Therapeutic Effect in Infants and Children with Hypochromic Anemia

KAISA LÄPINLEIMU AND RUTH WEGELIUS

Aurora Hospital, Helsinki, Finland

IN the iron chelate compound ferric sodium ethylenediaminetetraacetate,* the trivalent iron is firmly bound in an acid milieu. The compound dissolves readily in water, and it is not possible to demonstrate ionized iron with the customary chemical reactions. It is thus possible to produce pharmaceutical preparations with this compound that are suitable for pediatric use, since infants have no taste of iron and the preparation does not discolor the teeth. Theoretically, iron should not be released in the acid gastric juice but first in the alkaline milieu of the intestine. Will and Vilter[1] showed with radioctive iron that some of the ferric sodium ethylenediaminetetraacetate is split in the gastrointestinal canal and that the iron is absorbed in ion form and utilized in the synthesis of hemoglobin. Since all the iron is not released, the gastrointestinal disturbances, common as side effects of iron therapy, could be expected to be trifling. Ferric sodium ethylenediaminetetraacetate is of further interest in that it is a trivalent iron compound. It has, in fact, been held generally, ever since the investigations of Starkenstein and his colleagues in the 1920's, that only orally administered iron preparations that contain bivalent iron are of therapeutic significance in iron-deficiency anemia.

Ferric sodium ethylenediaminetetraacetate was tested by Wegelius[2] in infants and children with anemia. These preliminary clinical experiments proved the substance to have a distinct antianemic effect in hypochromic anemia. It also appeared that gastrointestinal disturbances were slight. This positive result encouraged further study. Iron absorption experiments were carried out according to Jasiński,[4] and clinical trials were continued, accompanied by serum iron determinations before and after therapy. The preliminary results were reported in 1957, by Läpinleimu and Wegelius.[3]

IRON ABSORPTION TESTS

The study consisted of 18 children with iron deficiency anemia, aged 12 months to 13 years. Table I shows the age, hemoglobin content, mean corpuscular hemoglobin content, serum iron values, and other delevant data. The diagnosis of hypochromic anemia was common to all the children. The hemoglobin values varied between 5.0 and 9.6 Gm./100 ml. (mean, 7.0 Gm./100 ml.). The mean corpuscular hemoglobin values varied between 13 and 24 $\mu\mu$g. (mean, 18 $\mu\mu$g.); and the serum iron values varied between 26 and 105 μg./100 ml. (mean, 72 μg./100

* The trade name of Oy Medica Ab for ferric sodium ethylenediaminetetraacetate is Plexofer.

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TABLE I

Hematological Data of 18 Children on whom Iron Absorption Tests were Performed

Patient no.	Age	Hemoglobin. Gm./100 ml.	Mean corpuscular hemoglobin. μμg.	Serum iron. μg./100 ml.	Remarks
1	14 mo.	7.5	17	93	Megacolon; acute infection
2	7 yr.	5.0	15	53	
3	17 mo.	7.1	15	83	
4	13 mo.	9.6	20	85	
5	12 mo.	6.7	19	83	Convalescent post pneumonia
6	8 yr.	8.3	24	99	Convalescent post acute infection
7	16 mo.	6.6	16	68	Weight at birth, 1940 Gm.
8	13 mo.	7.7	19	68	
9	13 mo.	6.6	14	61	Weight at birth, 1830 Gm.: morbilli
10	12 mo.	8.9	18	87	Weight at birth, 1500 Gm.; convalescent post acute infection
11	13 yr.	5.1	18	26	Thrombocytopenia; metrorrhagia
12	13 mo.	6.6	15	96	Weight at birth, 2300 Gm.: morbilli
13	16 mo.	6.7	13	66	Convalescent postotitis
14	20 mo.	6.7	14	58	
15	16 mo.	7.8	17	102	
16	8 yr.	7.6	21	105	Acute tonsillitis; epistaxis
17	3 yr.	7.5	16	40	
18	3 yr.	5.5	14	94	Convalescent post acute appendicitis

ml.). Ten patients were given ferric sodium ethylenediaminetetraacetate; the remaining 8 served as controls and received ferrous gluconate,† which had been found positive in effect by Jasiński[4] in similar iron loading dose trials.

The method and dosage of Jasiński[4] were employed. The dose of the trivalent compound was 152 mg.; the bivalent drug dose was 132 mg. The serum iron level was determined on a fasting stomach in the morning, following which the iron dose was administered orally and the serum iron value determined again one and three hours later. The serum iron values were determined according to the method of Kingsley and Getchell.[7] The normal values with this method are 125 to 230 μg./100 ml. for men and 120 to 200 μg./100 ml. for women. Figure 1 shows that the trivalent ferric chelate is absorbed with great readiness and that the rise in the serum iron values is of the same magnitude as that obtained with the bivalent ferrous gluconate. There was no theoretical or demonstrable difference between the children responding with high absorption values and the children responding with low values.

† The trade name of Sandoz A.G. for ferrous gluconate is Ferronicum.

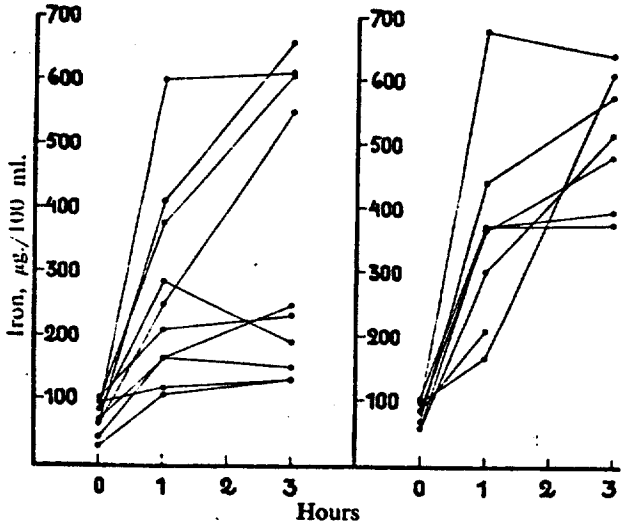

FIG. 1. Comparison of serum iron levels in 10 patients after oral administration of ferric sodium ethylenediaminetetraacetate (*Left*) and ferrous gluconate (*Right*).

THERAPEUTIC TRIALS

Ferric sodium ethylenediaminetetraacetate was used routinely in the ward for the treatment of different types of hypochromic anemia. Of 402 children given this drug, 251 were premature infants about 1 month old. The younger children were given 19 mg., while the older children received 38 mg. of the drug, two to three times a day. As usual with oral administration of iron, the initial dose was small and then increased gradually. The general clinical impression was that the therapeutic effect of the preparation in question was good and fully comparable with that of other iron compounds employed previously. In 15 cases of essential hypochromic anemia, the antianemic effect was established by serum iron determinations before and after treatment, besides the assessment from routine examinations (fig. 2). The results confirmed the clinical impression that ferric sodium ethylene-

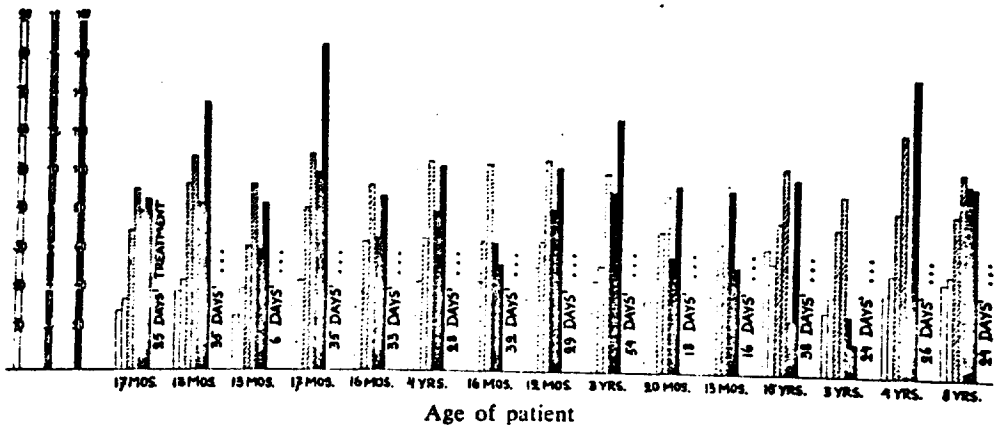

FIG. 2. Serum iron determinations in 15 patients before and after treatment with ferric sodium ethylenediaminetetraacetate. □, mean corpuscular hemoglobin content, μμg.; ▨, hemoglobin content, Gm./100 ml.; and ■, serum iron, μg./100 ml.

diaminetetraacetate administered orally is an effective antianemic agent. Of the 402 patients given this drug, 29 (7.2 per cent) had to discontinue treatment because of gastrointestinal side effects of varying degrees of severity.

DISCUSSION

Both the increase in the serum iron content following oral loading doses and the therapeutic experience obtained with ferric sodium ethylenediaminetetraacetate show that for hypochromic anemia, this trivalent iron compound is equal to ferrous gluconate, an iron compound well known and extensively used in Europe. A possible explanation is that the iron of the trivalent complex is rapidly reduced in the stomach and the upper portion of the intestinal canal to a soluble and ionizable bivalent iron.

An observation previously made by Jasiński[4–6] with ferrous gluconate (but not seen so clearly in connection with the ferrous gluconate loading doses in this study) was demonstrated clearly in connection with the oral administration of ferric sodium ethylenediaminetetraacetate. In some of the patients, the serum iron concentration rose after administration of the trivalent drug to very high values (500 to 700 μg./100 ml.); in others, considerably more moderate increases (100 to 200 μg./100 ml.) were established. Jasiński discussed this phenomenon but could account for these striking differences no more than we can.

We have thus, as Will and Vilter[1] showed with adult patients, been able to show in pediatric patients with anemia that the trivalent iron compound is absorbed equally well and that it gives an identical therapeutic response as equivalent quantities of iron in bivalent compounds. In pediatrics, ferric sodium ethylenediaminetetraacetate seems to offer a decisive practical advantage, in that the strong linkage of iron in complex bonds enables the preparation of water solutions free of ionized iron. Thus, palatable pharmaceutical preparations that do not discolor or otherwise harm the teeth can be easily prepared. Also, the incidence of gastrointestinal disturbances was small (29 out of 402 cases). Herridge[8] recently reported diarrhea as a prominent symptom when this preparation was used in children and adolescents. However, he employed a considerably larger dose than that used by us, and he presumed that a smaller dose would give a therapeutic effect with fewer side effects. This was, in fact, confirmed in Wegelius's investigation[2] and in the present study.

SUMMARY

Iron absorption tests were performed with ferric sodium ethylenediaminetetraacetate and ferrous gluconate. The dose of the trivalent compound was 152 mg.; the bivalent drug dose was 132 mg. Both were administered orally to 18 infants and children with iron-deficiency anemia. The increase in the serum iron value was of the same range with the two preparations.

Routine treatment of 402 children showed that ferric sodium ethylenediaminetetraacetate given orally had the same antianemic effect as the ferrous compounds

used previously. The incidence of gastrointestinal side effects was small (7.2 per cent). From the pediatric standpoint, this drug, because of its firm binding in chelate, has the advantage of enabling the manufacture of palatable water solutions free of ionized iron.

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We are indebted to Oy Medica Ab and Sandoz A.G. for supplying ferric sodium ethylenediaminetetraacetate and ferrous gluconate, respectively.

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Some Therapeutic Implications of Ferrous Sulfate-Ascorbic Acid Mixtures[1,2,3,4]

PAUL R. MCCURDY, M.D.,[5] AND RAYMOND J. DERN, M.D., PH.D.[6]

THE CURRENT STANDARD THERAPY for iron-deficiency anemia is ferrous sulfate administered orally three times daily as tablets, each containing approximately 60 mg of iron (1). While this program usually provides a maximum rate of hemoglobin regeneration (2), it is not effective in filling iron stores after the anemia has been corrected (3), unless administration is continued for a very long period of time (4). This, coupled with the observation that ferrous sulfate may induce gastrointestinal disturbances in some individuals and that patients frequently do not take multiple daily dose medications as prescribed (5), has resulted in a continued search for a better therapeutic regimen. Unfortunately, many claims have been poorly documented and a number of clinical studies affected by weak experimental design due to lack of controls and inadequate exclusion of potential sources of bias. However, certain facts are clear. In careful studies, Brise and Hallberg (6) found no compound of iron to be absorbed better than ferrous sulfate. Furthermore, when ascorbic acid was added incrementally, they demonstrated an augmentation of iron absorption from aqueous solutions of 30 mg of iron as ferrous sulfate. With 500 mg of ascorbic acid, iron absorption was increased by approximately 50% (7).

The present study was undertaken to define more clearly the effect of ascorbic acid on the absorption of ferrous sulfate. Accordingly, it was proposed to determine *a*) whether or not the enhancing effect of ascorbic acid on iron absorption occurs at high enough levels of iron dosage (in both tablet and controlled release forms of ferrous sulfate) to indicate the addition of ascorbic acid to therapeutic iron compounds; and *b*) whether or not the effect is great enough to permit the formulation of a single daily dose regimen for iron therapy.

MATERIAL AND METHODS

The subjects were normal Negro and Caucasian male prisoners who volunteered for the study. All had hematocrit values of 40–50%, and no abnormal hemoglobin disease as shown by screening cellulose acetate electrophoresis. (Some subjects did have sickle cell trait or hemoglobin C trait and one was later found to have thalassemia minima.) All had a transferrin saturation greater than 15%, generally excluding iron deficiency (8). Insofar as possible, subjects undergoing the same comparison had similar serum iron values. One subject donated blood 2

[1] From the Georgetown Medical Division, D. C. General Hospital, Washington, D.C.; the Department of Medicine, Loyola University Stritch School of Medicine; and the Department of Hematology, Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Illinois.

[2] Supported (in part) by National Institute of Arthritis and Metabolic Diseases Research Grants AM-02823, AM-09919, and AM-00946.

[3] An abstract of part of this work has been published: *Am. J. Clin. Nutr.* 20: 367, 1967.

[4] Reprint requests should be sent to Paul R. McCurdy, M.D., D. C. General Hospital, Washington, D. C. 20003.

[5] Associate Professor of Medicine, Georgetown University School of Medicine, and Medical Officer (Hematology), D. C. General Hospital. [6] Professor of Medicine, Loyola University Stritch School of Medicine, and Attending Physician, Cook County Hospital.

weeks before the study; no others were blood donors during the study or the 2 months previous to it. After the objectives and techniques of the experiment were explained, each subject indicated in writing his willingness to participate.

The absorption of one iron formulation was compared with that of another by the method of Brise and Hallberg (9) except that the activities of the ^{55}Fe and ^{59}Fe were measured by liquid scintillation counting (10). Briefly, each daily dose of one preparation was labeled with approximately 30 μc ^{55}Fe, and of another with 3 μc ^{59}Fe. Except as indicated, each medication was given as a single dose on alternate mornings about 3 hr after breakfast and 1 hr before lunch. When a medication was to be given three times daily, approximately the same time relationship to meals was maintained. Sundays, and sometimes Saturdays, were omitted; however, the full course was always given. In each comparison set, some subjects received the ^{55}Fe-labeled formulation on the 1st day whereas others took the ^{59}Fe-labeled preparation first. Ten days after the last dose of tagged iron, a blood sample was obtained. The ratio of ^{55}Fe activity to ^{59}Fe activity in this sample is the ratio of absorption of the two dosage forms. Three control studies were performed in which the absorption of one compound labeled with ^{55}Fe was compared with that of the same compound labeled with ^{59}Fe. The ^{55}Fe:^{59}Fe ratios were 1.11, 1.00, and 0.92. This demonstrated the range of experimental variation to be expected and showed also the similarity of absorption of both isotopes.

Preparations to be administered as aqueous solutions were preserved by lyophilization until used and were dissolved in 20 ml 0.005 N HCl just before being taken. The one labeled "without ascorbic acid" contained 10 mg of ascorbic acid to maintain the iron in the reduced or ferrous form. Brise and Hallberg (7) found that 50 mg ascorbic acid had no measurable effect on the absorption of iron with this technique. Each vial or tablet of ^{59}Fe-labeled medication was assayed directly before shipment. Since each ^{55}Fe preparation could not be so assayed because the X-ray emanation of this isotope is too weak, other methods had to be used to effect quality control. In some instances random samples from each lot were assayed or saved for standards. In others, a trace of ^{59}Fe was added to the batch so that individual tablets could be assayed to insure uniformity. A similar procedure was used for the preparation of the "controlled or delayed" release tablets except that the labeled ferrous sulfate was mixed with the plastic vehicle[7] before pressing into a tablet. Where ascorbic acid was included in the plastic embedded tablet, two types of preparation were made: in *A*, the ascorbic acid was mixed with the ferrous sulfate and the plastic to form a homogeneous mixture before pressing; and in *B*, the ascorbic acid was added to the outside of the tablet as a layer. Except where indicated, each tablet of a comparison pair contained the same amount of iron.

The absorption ratio data for each experiment were tested against the hypothesis that the mean was equal to 1. Since the values for both numerator and denominator would be normally distributed, the ratio would follow a Cauchy distribution (11). The hypothesis was tested by computing the regression line of numerator on the denominator of the form $y = a + bx$ and testing for $a = 0$ and $b = 1$. If so, $y/x = 1$ and the hypothesis cannot be rejected. For comparison of two sets of experiments, equality of the coefficients of the two regression curves was tested.

RESULTS

To determine the optimum quantities of ferrous sulfate and ascorbic acid for best iron absorption, pilot studies were done with aqueous solutions. These data are shown in Table 1. With doses of iron varying from 15 to 120 mg, ascorbic acid, in 200- or 500-mg amounts, potentiated the absorption of iron. With all quantities of iron, 500 mg ascorbic acid were better than 200 mg.

While ascorbic acid potentiated the absorption of the largest amount of iron used, further studies were limited to tablets containing 105 mg iron as $FeSO_4$ embedded in a plastic matrix with 500 mg ascorbic acid; due to size limitations, this ap-

[7] A copolymer of methyl acrylate with methyl methacrylate, prepared essentially as Fero-Gradumet/500 through the courtesy of George H. Berryman, M.D., Medical Director, Abbott Laboratories, North Chicago, Ill.

TABLE I

Comparative iron absorption from aqueous solutions containing various amounts of ferrous sulfate and ascorbic acid

Iron, mg	Iron-Absorption Ratio		
	Ascorbic acid, mg		
	200/10	500/200	500/10
15	1.42	1.80	2.00
	2.14	1.33	1.74
30	1.17	0.76	1.70
	1.31	1.54	1.00
60	1.79	1.55	2.67
	1.16	1.71	2.36
120	0.83	1.29	0.92
	1.17	1.25	2.36
Mean	1.37	1.40	1.84
Range	0.83–2.14	0.76–1.80	0.92–2.67

proaches the largest quantity of these two compounds that can be combined in a single tablet which is clinically acceptable (G. H. Berryman, personal communication). The results of the absorption studies are given in Table II. The addition of ascorbic acid nearly doubles (× 1.88) the absorption of iron from ferrous sulfate embedded in the plastic matrix (experiment I). Furthermore, it appears to make little difference whether the ascorbic acid is present with the iron throughout the release of iron, or whether the entire amount of ascorbic acid is available immediately while only the iron release is delayed (experiment II and intercomparison of means of experiments III and IV). Plastic-embedded ferrous sulfate with ascorbic acid is better absorbed than plain tablets of

TABLE II

Comparative iron absorption from tablets

	Iron-Absorption Ratio						
	Expt. No.						
	I	II	III	IV	V	VI	VII
	Type						
	FeG5B / FeG	FeG5B / FeG5A	FeG5A / FeS	FeG5B / FeS	FeG5B / FeS-5	FeG / FeS	FeG5B / F3
	1.05	0.59	1.22	1.48	0.89	0.54	0.65
	2.17	1.43	3.00	1.67	0.79	1.20	0.42
	2.11	1.06	1.29	1.59	1.21	0.91	0.75
	1.56	1.76	0.79	1.55	0.82		0.90
	1.93			1.25			
	2.46			2.27			
				1.41			
				1.07			
				1.50			
Mean	1.88	1.21	1.57	1.53	0.93	0.88	0.68
Range	1.05–2.46	0.59–1.76	0.79–3.00	1.07–2.27	0.79–1.21	0.54–1.20	0.42–0.92

FeG5B = resin-embedded $FeSO_4$ (105 mg Fe) with 500 mg ascorbic acid as layer.

FeG = resin-embedded $FeSO_4$ (105 mg Fe).

FeG5A = resin-embedded $FeSO_4$ (105 mg Fe) with 500 mg ascorbic acid mixed homogeneously with iron.

FeS-5 = $FeSO_4$ (105 mg Fe) with 500 mg ascorbic acid.

FeS = $FeSO_4$ (105 mg Fe).

F3 = $FeSO_4$ (60-mg Fe tablets given 3 times daily).

rous sulfate (experiments III and IV), ai when both modes of administering an incorporate ascorbic acid, the absorp- on is simila (experiment V). Similarly, hen neither the plastic-embedded iron nor the plain tablets contain ascorbic acid, he absorption is the same (experiment VI). When these comparisons were tested atistically as indicated, the differences noted above were found to be significant.

Since one of our objectives was a medi- ation that would provide adequate ther- py when administered once daily, plastic- mbedded ferrous sulfate (105 mg Fe) with scorbic acid administered once daily was ompared with standard ferrous sulfate tablets (60 mg Fe) administered three times daily (experiment VII). The absorption of ron from one plastic-embedded dose of ferrous sulfate with ascorbic acid (105 mg Fe) was 0.68 the absorption of three doses of plain ferrous sulfate (total of 180 mg Fe).

DISCUSSION

These studies extend the observations of others by demonstrating that the enhance- ment of iron absorption by ascorbic acid persists with amounts of iron up to 120 mg (aqueous solution) or 105 mg (tablets). Hence, formulations containing ascorbic acid might permit adequate therapy of iron-deficiency anemia with less frequent administration of medication than is now practiced. These studies were done in nor- mal individuals, and the results may not be the same in iron-deficient subjects who are more avid for iron. However, a valid basis is laid for therapeutic trials com- paring a single daily dosage with conven- tional therapy, and such studies are in progress. Even if the potentiation of iron absorption by ascorbic acid found in nor- mal volunteers is not borne out in the iron- deficient subject, the findings may have clinical bearing. Once iron deficiency is corrected, iron absorption decreases and prolonged administration of iron is re- quired to produce significant storage iron (3, 4). Ascorbic acid containing prepara- tions should be better in this regard. As with any iron preparation, clinical judg- ment must be exercised. It has been re- ported that prolonged use of standard iron medication over a period of years can lead to a pathological accumulation of storage iron (1). Therefore, there exists the theo- retical possibility that the better absorbed ascorbic acid-iron preparations could result in a faster accumulation of iron in such individuals.

Considerable interest in "delayed or sustained release" formulations has been generated by evidence that patients often fail to take medication as prescribed, with the proportion of omitted doses increasing as the number of daily doses increases (5). However, delay of iron release until part of it has passed well beyond the duo- denum, where iron is best absorbed, could only result in less absorption. Conse- quently, it is of interest that the plastic embedded ferrous sulfate preparation used in these studies was as well absorbed as standard ferrous sulfate tablets. Therefore, it seems reasonable to assume that this particular plastic embedding process does not delay the release of iron beyond the duodenum and that this preparation is not "sustained release" in the usual sense of the word.

The absorption of iron from a single daily dose of 105 mg iron with ascorbic acid was 0.68 times that of three 60-mg doses of plain ferrous sulfate given in 1 day. The apparently limited effect of ascorbic acid in this test is probably due to the fact that iron absorption does not in- crease linearly with dose but rather de- creases proportionally as the individual dose is raised. Nevertheless, the advantage gained by the administration of smaller divided doses only follows if the schedule is followed regularly without omission of any doses. However, the fact that the ab- sorption from a single dose is nearly 70% of that from the standard regimen forms a

reasonable basis for setting up clinical trials.

SUMMARY

Ascorbic acid has been shown to potentiate the absorption of ferrous sulfate in aqueous solutions, in standard tablet form, and in a plastic matrix. The potentiation increases with increasing doses of ascorbic acid up to 500 mg and holds with doses of iron up to 120 mg. The use of iron preparations containing ascorbic acid may permit the use of less frequent doses in the therapy of iron-deficiency anemia and may refill iron stores better than oral iron salts without ascorbic acid.

The authors wish to express their appreciation to Mrs. W. L. Hart for technical assistance in performing the radioiron assays.

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IRON ABSORPTION: EFFECT OF ANEMIA AND SUCCINIC ACID*

B.C. Mehta**, N.M. Purandare***, J.C. Patel****

Change in the serum iron after ingestion of iron tablets has been used to decide whether to give oral or parenteral iron.[4] Though serum iron is affected by several factors e.g. inflow from iron stores, outflow to stores and bone marrow, diurnal variations, etc. changes in serum iron following ingestion of iron provides an easy and reliable test of iron absorption. This report is a study of such a test in hematologically normal subjects and iron-deficient subjects.

MATERIAL AND METHODS

All tests were done on patients in the medical wards or hematology department of the King Edward Memorial Hospital. Patients were instructed not to ingest any food after 10.00 p.m. till the test was completed. Ingestion of water was not restricted. Blood was collected at 8.00 a.m. on the following morning for hemogram and determination of serum iron. Subsequent samples were collected at suitable intervals. Serum iron was estimated as described by King and Wooton.[5] The term anemia is used here to mean iron deficiency anemia with transferrin saturation below 15 per cent.

Group I:

There were 30 patients, 10 anemic and 20 non-anemic. Blood was collected at 8.00 a.m. after an overnight fast. A second blood sample was taken three hours later without administration of any iron. Serum iron level of the two samples was compared to see whether there were any spontaneous variations in serum iron.

Group II:

There were 65 patients, 35 anemic and 30 non-anemic. After collecting the fasting blood sample at 8.00 a.m., patients were given 150 mg. of ferrous sulphate powder and blood was collected again two, three and four hours later to determine which of these three samples yielded the highest serum iron values.

Group III:

There were 25 non-anemic subjects with no gastro-intestinal symptoms and 50 anemic subjects. Blood samples were collected in the fasting state and three hours after ingestion of 150 mg of ferrous sulphate powder.

Group IV:

There were 20 anemic subjects in whom absorption studies similar to group III were done. Few days later iron absorption studies were repeated with powder containing 150 mg ferrous sulphate and 50 mg succinic acid. Differences in iron absorption on two occasions in the same subjects were recorded.

* From Hematology Dept., King Edward Memorial Hospital, Bombay 12, India.
** Hon. Asst. Physician, K. E. M. Hospital, Bombay 12.
*** Prof. of Pathology, K. E. M. Hospital, Bombay 12.
**** Hon. Consulting Physician, K. E. M. Hospital, Bombay 12.
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RESULTS

Serum iron estimated on two blood samples taken at intervals of 3 hours from the same subject did not show significant variation (Table 1).

TABLE 1.

Mean serum iron at intervals of 3 hours.

No. of Subjects	Serum iron at 8.00 a.m. mcg./100 ml		Serum iron at 11.00 a.m. mcg./100 ml	
	Mean	S.D.	Mean	S.D.
30	62.9	41.1	70.5	40.8

Results of serum iron 2, 3 and 4 hours after ingestion of 150 mg ferrous sulphate show that maximum rise occurs at 3 hours (Table 2).

TABLE 2.

Mean rise in serum iron 2, 3 and 4 hours after 150 mg ferrous sulphate ingestion (75 subjects, 45 anaemic and 30 non-anaemic).

Serum Samples	Rise in serum iron (Mcg./100 ml.) Mean	S.D.
2 hours	95.2	90.8
3 hours	118.0	119.3
4 hours	92.5	121.7

In 30 non-anemic subjects, the mean rise in serum iron three hours after ingestion of 150 mg ferrous sulphate was 81.8 mcgm. per 100 ml. Eighteen subjects had a rise of less than 80.0 mcgm. per 100 ml and could be considered to have malabsorption of iron. In forty-five anemic subjects the mean rise in serum iron three hours after ingestion of 150 mg of ferrous sulphate was 157.6 mcgm/100 ml. Eighteen patients had rise in serum iron of less than 80 mcg./100 ml. Iron absorption increased with increasing severity of anemia (Fig. 1).

In twenty anemic subjects, there was a mean increase of 80.9 mcgm. in rise of serum iron 3 hours after iron ingestion when iron was given along with 30 mgm succinic acid (Table 3 & Fig. 2).

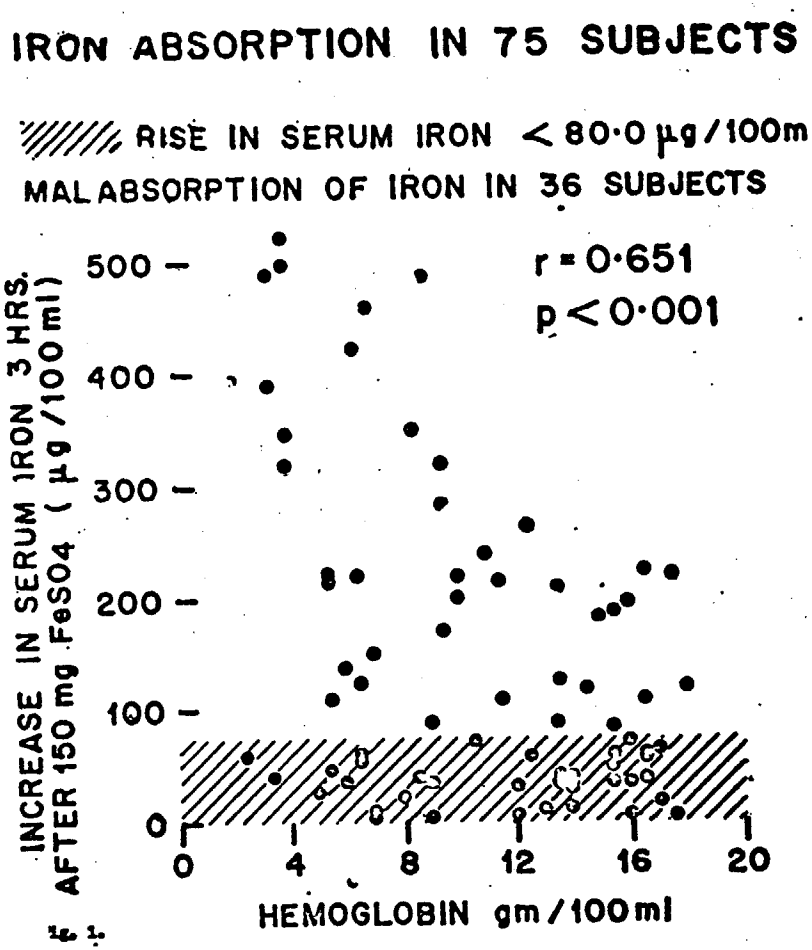
TABLE 3.

Effect of succinic acid on iron absorption (20 patients)

Increase in serum iron 3 hours after ingestion of			
Ferrous sulphate 150 mgm		Ferrous sulphate 150 mgm +succinic acid 50 mg.	
Mean	S.D.	Mean	S.D.
151.9	128.1	232.8	137.8

DISCUSSION

Though diurnal variations in serum iron are known to occur, serum iron values at intervals of three hours as determined in the present study do not vary much. These variations (mean 7.12 mcg.) are much smaller than the rise in serum three hours after ingestion of 150 mg of ferrous sulphate in non-anemic (mean rise 81.8 mcg.) subjects. Based on this observation, a rise of less than



80.0 mcg. was considered as an evidence of iron malabsorption. Israels and Simmons[4] considered increase of less than 100 mcg. as indicating malabsorption. However they administered 105 mg of elemental iron for the test. Eighteen out of thirty non-anemic subjects showed malabsorption of iron. In 45 anemic patients there were 18 patients with malabsorption of iron. Iron absorption increased with increasing severity of anemia.

It has been reported that the average Indian diet contains a liberal supply of iron.[6,7] Despite this, the incidence of iron deficiency amongst Indian people is high. This has been attributed to lack of availability of iron due to high phytate content of predominantly cereal based food.[1,7] Increased loss of iron through perspiration in tropical climates has been suggested as the cause of a high prevalence rate of iron deficiency.[1] The present study suggests yet another explanation, namely, malabsorption of iron, for the high incidence of iron deficiency.

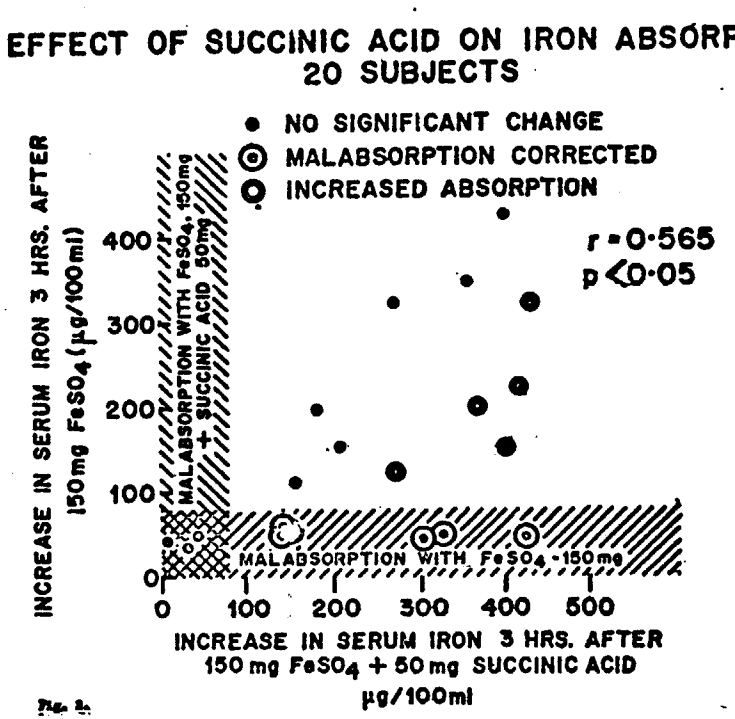


Fig. 2.

Response to oral iron does not rule out malabsorption because the therapeutic dose of iron is usually much higher and is administered several times a day.

Administration of 50 mgm succinic acid alongwith 150 mgm ferrous sulphate led to improvement of iron absorption. Nine patients had malabsorption of iron when they were given only ferrous sulphate. Six of these patients had improved absorption of iron when succinic acid was given along with ferrous sulphate. Thus addition of succinic acid to an iron salt improves the chances of success of oral therapy. Brise and Hallberg[2] have shown by a series of experiments using double isotope technique that succinic acid administered with an iron salt increases the iron absorption by increasing the cellular metabolism.

SUMMARY

Increase in serum iron 3 hours after ingestion of 150 mgm ferrous sulphate was used to assess the iron absorption. Increase of less than 80 mcg./100 ml in serum iron level is considered to indicate malabsorption. Malabsorption of iron was found in 18 out of 30 non-anemic subjects and 18 out of 45 anemic patients. Iron absorption increased with increasing severity of anemia. Succinic acid corrected malabsorption in 6 out of 9 cases.

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A COMPARATIVE EVALUATION OF VARIOUS IRON PREPARATIONS

L. I. Mikhaylova

Hemotherapeutics Clinic (Head - Prof. P. M. Al'perin), Central Institute of Hematology and Blood Transfusion (Director - Docent A. Ye. Kiselev), Moscow

Iron is an agent for the pathogenetic treatment of iron-deficient anemias; consequently, the use of such (iron) preparations - which can enter the gastro-intestinal tract in the most accessible (ferrous) form, be quickly ionized, be resorbed by the intestine wall, enter into the plasma, and from there, into the storage organs - has great importance.

It may be conjectured that the effectiveness of iron preparations, according to the degree of the increase of the serous iron level during a charge of one or another (iron) preparations, reflects the degree of resorption of a given drug and (the degree) of its entry into the storage organs (Heilmeyer and Plotner; Dreyfus and Schapira; M. S. Dul'tsin, Ts. D. Makarovskaya, et al.).

Studies on the fluctuations of serous iron content in practically healthy people have shown an insignificant increase of its level after an (iron preparation) charge. Following phlebotomy in the presence of an iron deficit in the organism, a significant rise in the level of serous iron after an (iron) preparation charge is noted. With the adequate saturation of the storage organs by iron following conducted therapy, the serous iron level increase during an iron charge becomes insignificant once again.

It is certain that through the intake of one or another iron preparations, a noticeable growth in the amount of iron in the serum is obtained; the systematic use of these preparations in rational dosages generally promotes an improvement in the composition of the blood.

In (medical) literature, insufficient attention is given to the comparative evaluation of the effect of various medicinal iron preparations on the fluctuation of serous iron in patients with iron-deficient anemias.

We undertook to investigate the effectiveness of those iron preparations which have received the greatest distribution in the medical practice. The problem (studied) is on (the use) of hydrogen-reduced iron, iron carbonate, iron malate, and also on a new preparation - iron ascorbate.

Serous iron was studied according to Barkan's method, before treatment, and at 3, 6, and 24 hours after the intake of one or another of the iron preparations. Into 74 patients with latent chlorosis and symptomatic chloranemia, 79 charges of hydrogen-reduced iron, 22 charges of iron carbonate, 8 charges of iron malate, and 10 charges of iron ascorbate were inducted.

The most effective (of the preparations) proved to be iron ascorbate: the serous iron level was significantly increased - (to) more than 200 mkg. (microgram) % - in all patients, at 3 hours after the intake per os of 1 g. (against an initial trace level of 28 mkg. %). A significant increase in the serous iron level was also observed in a portion of the patients after 24 hours which was not noted during charges of the other preparations. This may be due to the more complete resorption of a given preparation from the intestines. Hydrogen-reduced iron, of which the serous iron level was significantly increased in 61 of 79 charges, stands in second place as to effectiveness.

In patients with latent chlorosis, intakes of iron carbonate, also in the amount of 1 g., hardly produced an increase in serous iron concentration. This preparation gave a better effect during symptomatic chloranemia. During the intake of iron carbonate, serous iron content increased to 120-150 mkg. % in only four of 22 patients, and in (another) 4 -

higher than 200 mkg. %. The specified persons suffered from chronic posthemorrhagic anemia with constant normal gastric secretions.

The least effective (of these preparations) proved to be iron malate, even in large doses (10 g.). The serous iron level was increased to 154 mkg. % during a charge of this preparation in only 1 of 8 patients; consequently, the preparation was excluded from further study.

COMPARATIVE ASSESSMENT OF VARIOUS IRON PREPARATIONS

L. I. Mikhailova

Summary

Various iron preparations, most common in the medical practice, are compared. The assayed serum iron level following loadings with these prepartions in 74 patients suffering from iron-deficiency anemias indicated iron accorbate te be the most affective one, which in all cases yeilded a considerable rise in the serum iron level. This was also observed in the majority of patients after taking hydrogen reduced iron. The smallest increase in the proportion of serum iron was noted following administration of iron carbonate, while the iron maleate effect proved to be almost nil.

61989j Comparative evaluation of various iron preparations. L. I. Mikhailova (Tsentr. Inst. Gematol. Pereliv. Krovi, Moscow). *Sov. Med.* 30(6), 51-2(1957)(Russ). Fe reduced by H, and its carbonate, malate, and ascorbate, were tested in 74 patients with Fe-deficient anemias. The most rapid and intense increase of blood serum Fe followed after administration of Fe ascorbate and pure reduced Fe, whereas the carbonate was without effect; the carbonate proved suitable for the treatment of symptomatic chloranemias. Fe malate (even in doses of 10 g.) was totally ineffective. Miloslav Kalab

INVESTIGATIONS ON IRON METABOLISM IN PIGS

BY F. MOELLER

The role of iron in many enzymes and in hemoglobin and myoglobin for cell respiration and for speedy transport to the tissues is well known. Major traits fo iron metabolism have further been unveiled in the discovery and research of deposit forms of iron ferritin and the iron transporting proteins in plasma transferrins. Many investigations have only shown that secretions of absorbed iron are fairly small, so small that they need only account for the amount of iron in relinquished cells. The consequence of the aforementioned condition is that the organism, in order to hinder the reserve iron from increase, must regulate its intake of iron (10). This regulation further permits that larger amounts of iron can be absorbed if the need increases (3). Such an increased need is found under normal physiological conditions in pregnant and in growing individuals. The processes that the regulation is based on, and which is of central importance in iron metabolism, is not known in spite of innumerable investigations.

With respect to our domestic animals, a clarification of the processes in the normal iron metabolism has noteworthy importance in the pig in that the pig, at birth, regardless of the extent of iron supply to the sow during pregnancy (16), has buy a limited reserve of iron, and therefore rapidly becomes dependent upon the amount of iron it can obtain from its enviroment. As the sow milk's iron content is fairly scant, and clearly doesn't cover the pig's requirements (16), iron supplement of one form or another becomes necessary. Discrete parenteral iron therapy, is doubtless, of great value in the treatment and prevention of sucking-pig anemia, reaching no better understanding of the iron metabolism processes with this treatment, a knowledge whereby a rational therapy of oral supply of iron could be based. These observations have motivated, in the following investigative descriptions by which means the methods of experimentation with the extent of iron absorption in sucking-pigs have been prepared, and how influence of experimenta factors on the iron absorption are attempted to be explained. The influence of ascorbic acid has been researched where it is partly known that ascorbic acid plays a part in the transfer of iron (8); partly that trivalent form, thus a reduction vehicle could promote absorption (17). One experiment is further carried out where absorption in sucking-pigs of experimental age have been researched; herewith, the need and thereby the absorption varies with age. In the last-named experiment, trivalet iron is used, while a parallel experiment in which a comparable iron combination was used will not be more closely

described as the research pigs acquired diarrhea during several periods. In addition, two pigs have been researched for absorption of a complex iron combination (fructose iron), and in one is the action of the absorption of a parenterally supplied iron (dextran iron) attempted to be illustrated.

Materials and Methods

Pigs of Danish origin are used in the experiments--in experiment No. IV, the pig species died. In each instance, there is mention of balance experimentation where separation in the feces and urine of a measured dose of radioactive iron has been determined. Supplementally, the activity in the blood has been measured. The collection was made in metal screen net cages protected with aluminum-bronze. The cages were placed sunken over a slanted bottom of plastic slopes. The urine, via an off-flow formed in the lower corner of the floor's lower edge, was collected in plastic bottles through a filter. The screen cages had the following dimensions: 70 cm long, 50 cm high, and 30 cm wide. The plastic slopes, in all, 6 arranged in 2 units, were placed 40 cm above the floor on metal frames in a small room wich was enclosed with a plastic sheet, there, however, to allow ventilation not reached by the air. The room was heated by heating lamps hung over the cages and by an electric heating oven. The temperature was maintained at about 27? C. In the final experiment, which will not otherwise be referred to, a thermostat regulated electric heating oven was used. A suitable humidity (relative humidity, 60 C) was reached by placing a dish of water in the room. Beyond the usual cleaning procedures, it was not attempted to avoid iron defilement in the cages during the collection.

In the foddering, which took place seven times a day: at 6 and 9 A.M., 12 noon, 3,6, and 9 P.M., and 12 midnight, either sow's milk or a sow's milk substitute preparation was used. In experiments I and II, sow's milk*which had previously been frozen, was good for three months at the most. In experiments III and IV, Laktal (blue Laktal, Kemovit) was used without mineral supplements. One kilogram of this was mixed with six liters of butter-milk and one liter of approx. 80 C warm water. In experiment No. V, a mixture is used which, in contrast to the mixture with Laktal (80% ground oats), is exclusively made up of milk products:

Eledon (Nestle, sour half skimmed milk, warm-air-dried)
Eledon : fats 14%, Protein 30.8%, Lactose 40%,
Mineral salts 6.5%, Lactic Acid 4.7%,
Water 4.0%)..... 200 g
Whipped cream..... 80 g
Water, upto.....1000 ml

All the pigs spent at least two days with the sow before synthetic nourishment was begun. The milk, which the pigs

*Made available through the Research Laboratory's department for experiments with pigs.

received from a small plastic nipple (a pierced plastic bottle), was heated to about 30°C before the feeding.

All the iron preparations were administered through a stomach tube (rubber catheter 3.5 mm in diameter) in a volume of 2 ml measured in a syringe, and the quantitative dosage was insured through flushing the tube with 3 ml of a 5% glucose solution. The fructose iron preparation was, however, administered in a volume of 5 ml, and the volume of the solution in experiment No. I was 1 ml.

The ferrous ascorbate was prepared in the following measurements (7). To a hydrochloric solution of ferrous chloride in a 250 ml centrifuge (flask), was added 1 n of sodium hydroxide to foment with bromide blue. After centrifugine, the supernatent was decantered off, and ascorbic acid was added in a proportion of 6 g ascorbic acid: 1 g iron to the precipitated ferrous hydroxide. Through stirring, the sediment dissolved in approximately 10 minutes, under continuous aeration and with the addition of a small amount of water, whereafter the solution was neutralized with 1 n NaOH. The blue-violet ferrous ascorbate, formed hereby, was precipitated with the indication to increase the solution to 20 volumes acetone. The precipitate was collected on a filter in a Büchner funnel and dried in a vacuum (freeze dried). The ferrous ascorbate contained, as a result, an iron analysis of 16% iron. It was dissolved immediately before use in distilled water.

Fructose iron (13) was prepared with a solution of ferrous chloride by placing 8 mol. fructose and sodium acetate, so that the molarity of the latter, in the solution's final volume, will be 0.1. Thereafter slowly neutralized and water was added, thus the solution contained approximately 10 mg iron per ml.

Iron ammonium citrate (11) was prepared by precipitating the iron ($FeCl_3$) in a hydrochloric solution with 1 n sodium hydroxide. After centrifuging and decantering of the above liquid, added to the contents was citric acid (monohydrate) in the proportion 4 g citric accid: 1 g iron, plus a small amount of water, whereby the contents, while being stirred in a water bath at 60°C, was partially dissolved. Thereafter neutralized slowly with concentrate ammonia water, whereby a completely clear solution resulted. Water was added to the solution to reach a content of about 20 mg. iron per ml.

Analyses. Guiding analyses of iron in feces and urine and iron analyses of milk preparations and iron preparations were conducted with a modification of Positano's and Weisel's method of determining serum iron with bathophenanthrolin (12). The weighed feces was homogenised manually with a measured amount 6 n hydrochloric acid. Of the homogenised manure, 0.5-1 g was removed for evaluation. Of the urine, the col-

lected amount or 50 ml. In experiment No. V, where the manure from six days was mixed, the fine separation was accomplished with water in a motor-driven homogeniser. After treating the material with sulphuric acid and hydrochloric acid, approximately 10 ml of redistilled water and 2 ml concentrated hydrochloric acid were added to the evaluation flask, whereafter it was heated to the boiling point, such that a possible sediment was dissolved. After quantitative transferral to a measuring flask and the addition of redistilled water to the mark, it was mixed, whereafter a quota portion containing from 0.5-5 micrograms of iron was removed. This was concentrated with ammonia water to a fomentation with methyline blue. Hereafter the measurement procedures were as described by Positano and Wiesel (12), with the exception that the procedure with bathophenanthroline iron complex was made in 3 ml amylalcohol and not in 5.

The radioactivity in the samples was determined, after completion of the above described and removal of a quota, through removing the iron in it electrolically on a copper plate and measuring the activity of the deposited iron with a Geiger-Müller counter. The electrolyte cells are of the same type as those described by Peacock, et al. (11) and the advanced measurements are otherwise modified after Hallberg's and Brise's methods for determining of Fe^{55} and Fe^{59} in blood (5). The modifications are that the iron was not removed as ferrous hydroxide, but as ferrous sulphide with a weak base reaction. The ferrous hydroxide separations were discarded when it became evident that the iron in the urine samples were not separated satisfactorily in this measurement procedure. The ferrous sulphide separation was partially based on the principles described by Stokes and Cain (15).

The chosen quota was introduced in a 250 ml centrifuge flask. If the chosen volume was less than 10 ml, water was added to 10 ml. To this was added: ¼ volume 50% potassium-sodium tartrate; 1 ml ferrous chloride solution (carrier iron 4.5 mg Fe per ml); 4 drops methyline blue (Ph.D.); and approximately 0.5 g hydroxylamine hydrochloride (fresh). After dissolving, and 5 minutes standing, 10 n sodium hydroxide were added, up to the exact point where the green colour switched to blue. A possible further separation with neutral reaction could be made to disappear again by making the solution sour and adding an additional 10 ml potassium-sodium tartrate. After the colour change, 0.5 ml 20% ammonium sulphide (10 drops) was immediately added. After mixing, standing 30 minutes under even shaking, wherewith the first established colloids of ferrous sulphide were completely separated out. The colloids can possibly be caused to separate with the addition of a few crystals of hydroxylamine hydrochloride. Thereafter, it was centrifuged

and most of the supernatent was carefully drawn off, whereafter the remainder of the supernatent was poured onto a small filter (2.5 cm in diameter, Whatman No. 541). At the most, 15 minutes later, the filter was introduced into the centrifuge tube, to which was added 1.4 ml 6 n sulphuric acid. Any precipitate on the sides of the tube was rinsed down with sulphuric acid and a little water (0.5-1 ml), whereafter the tube was introduced into a boiling water bath where the sulphur fumes and most of the water were evaporated. The solution was not so strongly concentrated that it attacked the filter. Thereafter, 2.5 ml 50% ammonium sulphate was added and the contents transferred quantitatively, with, in all, 40 ml measured ammonium oxilate, to an electrolyte cell. This was previously cleaned upon mounting with a copper disc (2.5 cm in diameter) which was cleaned by dipping in 30% nitric acid for about 30 seconds (until nitrogen was produced), rinsed with distilled water and dried with cotton wadding. After transferring the radioactive solution, the platinum electrode was placed in the -ell and the electrolysis was begun. Intensity of current and stress in 10 parallel, connected cells was noted after 30 minutes. With 0.4 amps per cell and 8 volts, the electrolysis was performed in 4 hours. After completion of the electrolysis, the cells were dismounted and the copper planchette was rinsed, first with distilled water and thereafter with 96% alcohol, whereafter it was dried with an electric lamp. The activity (Fe^{59}) in the deposited iron was then measured in a Geiger-Müller counter and the activity in the collected samples measured. The activity in the given dose of iron was determined by the same measurement as described above in that the dose was measured off by the same syringe that was used in preparation of the iron. Since the dose was used as a standard and was measured at the same time as the test samples, the per cent of the dose in these could be calculated immediately, wherewith they were automatically corrected for physical lapse of the radioactive iron. All the analyses, with the exception of a few urine and blood samples, are performed as double tests. It should be noted that in the analysis of the manure samples and blood samples from experiment No. I, the iron was deposited as ferrous hydroxide. In addition, the above method was used in a slightly modified form in experiments II, III, and IV. Here, the deposit of the colloidal ferrous sulphide was brought out with the introduction to the centrifuge flasks in about 5 minutes in a boiling-point water bath. After chilling, it was centrifuged and the sediment was washed once with a minimum amount of water (15-20 ml). Thereafter, it was centrifuged again and, after as completely as possible drawing off the supernatent, 6 n of sulphuric acid was added. In each case, the dose was treated in the same manner as the test samples. The activity was determined in all the samples, except for the urine samples, at a reading of about 10,000 counts. Due to the difficulty of manual homogenisation of the manure, some of the tests were performed on a basis of

guided analyses. The addition of the carrier-iron (4.5 mg) caused the deposited amount of iron to always lie between 4.5 and 5 mg of iron following the guided iron analyses of the samples. The measured activity in the standard samples did not vary where carrier-iron amounts were consistently 4.5 and 5 mg. With the exception of a few blood samples containing much radioactive iron, and samples where less than 1 ml was drawn, the activity in the blood was determined by analysis of 1 ml heparine blood. Calculation of the percent of the dose in the blood was performed by the following measurement, in that the blood volume was taken to be 60.5 ml per kilogram (own investigation):

$$\frac{\text{Activity in 1 ml blood} \times 100 \times 60.5 \times \text{full or ideal weight (kg)}}{\text{Activity of the Standard (Dose)}}$$

The hemoglobin tests were performed with the help of the cyanide-hemoglobin method.

Conditions of the Individual Experiments

I. Two nine-day-old pigs (litter 112), about 15 minutes after having received milk from the sow, received 21.5 mg marked iron (4μC Fe^{59}) as ferrous ascorbate. Manure and urine were collected thereafter 4 times a day for 8 days. The pigs were fed sow's milk (1.66 mg iron per liter) increasing from 410 to 740 ml daily. An average of 645 ml was received daily. For three days, in the middle of the experiment, no manure was deposited, probably due to the iron's constipating effect.

II. Five seven-day-old pigs (Nos. 4, 5, 6, 7 and 10, litter 119), after having been taken from the sow about 15 minutes earlier, received 45.5 mg of marked iron (approx. 11μC Fe^{59}) as ferrous ascorbate. Manure and urine were collected 4 times a day for 5 days. Shortly after administering, No. 10 vomited part of the dose, whereafter he was removed from the experiment. No. 4, three days before the experimental period was begun, and throughout, received 0.5 g ascorbic acid daily, divided into two doses. During the investigation period, the ascorbic acid was given in a 25% solution, before it, as tablets. No. 7 was treated 4 days and 2 days, for a balance each time of 75mg iron as dextran iron, given intramuscularly. The amount of sow's milk (1.05 mg iron per liter) was increased daily during the investigation period. The average daily amount of milk consumed was for No. 4: 275 ml, No. 5: 575 ml, No. 6: 480 ml, and No. 7: 385 ml. No. 4 got diarrhea the first evening of the balance period. On the third day the manure was again normal.

III. Six fourteen-day-old pigs (Nos. 1, 2, 3, 8, 11 and 12, litter 119) received fourteen-day-old 45.5 mg marked iron (approx. 11μC Fe^{59}) as ferrous ascorbate. The pigs had not been fed the last three hours before administration. The experimental period was predeeded by a two-

day period wherein the pigs adjusted themselves to the cages and the synthetic nourishment (Laktal). Manure and urine were collected twice a day for 4 days. During the pre-period and the experimental period, Nos. 3 and 11 received daily 0.5 g ascorbic acid divided into two doses. Likewise, Nos. 2 and 12 received 1 g daily. Half of the daily dose of ascorbic acid was given just before administration of the iron. In the first two days, the pigs received 70 ml Laktal (800 mg iron per liter) at each feeding, in the last two, 80 ml.

IV. Six eleven-day-old pigs (Nos. 1, 3, 8, 9, 11 and 12, litter 62), after a two-day pre-period in the research cages, received 55.4 mg marked iron (approx. 13μC Fe^{59}). The pigs had not been fed for the last 4 hours before administration. Nos. 1 and 3 received fructose iron, the rest received ferrous ascorbate. Nos. 8 and 12 were treated daily, during the pre and research period, with 0.5 g ascorbic acid divided into two doses. Half of the daily dose was administered just before the iron preparation. No. 9 was removed from the experiment after having vomited part of the dose. The research period lasted 5 days, wherein the manure and urine from one day was mixed. At each feeding, throughout the experiment, the pigs received 80 ml Laktal (8.9 mg iron per liter). No. 1 drank somewhat less on the second day of the experiment. The manure from this pig was, throughout the experiment, sparce, pastey, and a bit slimey on the surface.

V. Seven pigs (Nos. 1, 4, 5, 6, 8, 9 and 11, litter 101), after a two-day period in the research cages, received 44.2 mg marked iron (approx. 7μC Fe^{59}) as iron ammonium citrate at intermittent periods after the feeding. Nos. 1 and 8 were seven days old; Nos. 5 and 6, ten; Nos. 4 and 11, thirteen; and No. 9, seventeen days old at administration. The pigs were last fed three hours before. The experiment lasted seven days, during which the manure and urine were collected twice a day. The manure from the first six days was mixed, while the manure from the seventh day was used as a control by complete separation. The were fed increasing amounts of Eledon whipped cream (1.20 mg iron per liter) as follows: three-day-old pigs: 30-40 ml, seven-day: 40-50 ml, 13 day: 60-70 ml, and 21 day: 70-80 ml, at each feeding. For periods before the experiments, the pigs had had diarrhea. All the pigs were thus, at the age of 4 days, treated for diarrhea with 50 mg aureomycin. By the same method, Nos. 4, 11 and 9 were treated at ten days old, and No. 8 at eleven days old. None had diarrhea at the start of the experiments. No. 8 had diarrhea the last three days, and No. 4 the last day of the investigation period. Pigs with diarrhea received either no milk at all or only small amounts. Instead, salt water was given (1%).

Results and Discussion

Although the experiments are partially incomplete, in that the separation of the non-absorbed iron in many of the research pigs did not reach or come down to 1% in the last collection (12-hour period)(Table 1), and in that a number of the pigs have been sick, as seen in going through the results in Table 1, these are in agreement with the universally established criteria regarding iron metabolism. There is thus no question that the ferrous iron in these experiments is absorbed to a much larger degree than ferric iron (excluding Fructose iron). The reason that pig No. 11 in experiment No. III only absorbed about 5% of the dose can probably be found in the constituent factors in that the pig, without being clinically ill, did not thrive in the following months. That cannot, however, be the case for No. 12, in experiment No. IV, since this pig thrived the entire time. A beginning diarrhea which ceased upon administration of iron could possibly be the reason for the slight absorption in this case. In addition, it is thought, in agreement with other experiments on the influence of the size of the dose on the extent of absorption (3), that the absorption in experiment No. I has been larger (about double, in percent) than the absorption in experiment No. II, where twice as much iron was used in the dose. It is presumed here that the constipation experienced by the pigs in the middle of the first experiment did not increase the absorption in that this essentially takes place in the first part of the small intestine (duodenum) (4, 14), as the iron must have passed this time period.

The milk used has also influenced the absorption in that the pigs fed sow's milk (I and II) had absorbed more iron than the pigs that were fed Laktal, in which some of the iron was excreted in the intestinal canal (phosphate, fytin (9)). Of importance in this connection, it is also possible that the pigs in experiments I and II received milk shortly before administration of the iron, while the Laktal-fed pigs received milk 3 to 4 hours before administration. A mixture of iron with milk will, by increasing the dose's surface, be able to effect increased absorption.

Administration of dextran iron (No. 7, experiment II) has apparently not hampered absorption in the colon. On the other hand, it is seen that the activity in the blood has not been extensive in that the radioactive iron has competed with the much larger amounts of inactive iron with the incorporation into the hemoglobin (blocking action (2)). The pigs' hemoglobin percent rose, in the three days prior to the experiments, from 6.6% to 8.9%; this very active haematopoese has possibly effected the larger absorption (1).

In all the pigs that were treated with ascorbic acid, when disregarding the already mentioned pigs (11, experiment III and 12, experiment IV), an iron absorption is measured which is of the same amount or more than the control animals. This shows that it is undoubtedly the administration of the ascorbic acid just before the administration of the iron. The action of the constantly supplied amount of ascorbic acid is shown in Fig. 1, where the activity in the blood, for three weeks after administration of the iron, is given. The figures in parentheses are the absorptions measured measured during the experiment. It is seen that the concentration of radioactive iron rises sharply in the pigs that have received ascorbic acid, in that a plateau is reached after 5 to 7 days, while the comparable time in the control pigs is 10 days. If there is, herewith, discussion of reaction on the haematopoese, or the iron's mobility cannot be determined, but such a double action is possible. It is also seen in the table that the complex of iron with fructose (No. 3, experiment IV) is absorbed in the same volume as with the pigs that received ferrous ascorbate and ascorbic acid, and that the absorption is greater than with the other used iron sombinations (experiment V).

In experiment No. V, where the absorption of an iron combination was measured in pigs of varying ages, the influence of growth and the increasing iron requirements on absorption is shown; this was, contrary to expectations, decreased the older the pigs were. The distinctions are, meanwhile, not so great and the results overlap one another, depending possibly on chance variations. It should also be noted that the hemoglobin percent of these pigs was high, and that their weight hardly varied at all. Excretion of the marked iron in the urine of all the pigs was very slight, and those instances where it has been 0.1% of the dose or more can be due to mixing of the iron from the manure. In the table's second to last column, the amounts for the maximum measured activity in the blood are stated. The average of the two highest measured values, after having reached a plateau (Fig. 1) is used. These figures should be, if all the absorbed iron amounts were used for hemoglobin synthesis, in agreement with the balance experiment's results. There are, however, a few factors that can influence the irons' utilization for hemoglobin synthesis. Partly, the haematopoese can be decreased as in infections (6); partly, some of the iron will be used in the synthesis of other iron-containing combinations (myoglobin, etc.); a d partly, that numerous changes can be brought about through changes in the blood volume in connection with feeding and blood samples drawn. It is seen, however, in Fig. 1, that the picture of absorption in these two methods is decidedly largely the same. Supposedly, the activity in the blood can be used as a goal for absorption in similar experiments under various conditions of

research if the blood samples are taken frequently, infections in the pigs can be eliminated, and if the research animals, as in this instance, have very little or no iron deposits.

In spite of the technical differences connected with the balance experiment with the piglets, especially the artificial nourishment, the frequent intestinal upheavals, and problems with the quantitative collection of the manure, may result in some of the research pigs being removed from the experiments, these still give valuable information, also of practical value on the nature of iron metabolism.

It is therefore evident from this research that the best use of peroral iron is indeed reached by beginning early in giving the pigs small doses of ferrous salts at intervals of a few days. A reduction media can possibly be given at the same time and the iron, shortly after the pigs have received milk from the sow. Fructose iron, which seems to be absorbed to the same extent as a ferrous salt and ascorbic acid, will in practice be of great value as the solution of the complex is stabile. Further experiments with this combination are, however, necessary before conclusions can be drawn regarding its value in peroral iron therapy.

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Tabel 1. Oversigt over jernbalanceforsøg.
(Table of iron balance experiments).

Forsøg nr. (exper. no.)	Gris nr. (pig no.)	Ved forsøgets start (at start of exper.) alder dage (age days)	Hæmogl. (hemogl.) g %	Vægt kg (weight kg)			% af dosis (Fe^{59}) udskilt (% of dose (Fe^{59}) excreted) i gødning i alt (feces total)	i urin i alt (urine total)	i gødning sdt. dag (feces last day)	% af dosis resorberet (% of dose absorbed)	Max. aktivitet i blod % af dosis (max. activ. in blood % of dose)	Standardafv. på enkeltbestemmelse af Fe^{59} i gødning (stand. deviation on single determination of Fe^{59} in feces)
				1. dag (1. day)	9. dag (9. day)							$\delta i = \sqrt{\frac{\Sigma x^2}{2N}}$
I	1	9	7,8	2,47	3,82	Ferroaskorbat ≈ 21,5 mgFe	17,4	0,1	0,5	82,5		$= \pm 0{,}37\,\%$
	8	9	6,7	2,62	3,27	Ferroaskorbat ≈ 21,5 mgFe	26,3	–	1,9	73,7		
				1. dag (1. day)	4. dag (4. day)							
	4	7	6,1	1,96	1,76	Ferroaskorbat ≈ 45,5 mgFe 0,5 g askorbinsyre 57,6	57,6	0,1	2,2	42,3	14,2	
II	5	7	7,9	1,86	2,35	Ferroaskorbat ≈ 45,5 mgFe	50,7	< 0,1	7,0	(49,3)	29,4	
	6	7	6,7	2,08	2,47	Ferroaskorbat ≈ 45,5 mgFe	67,5	< 0,1	40,2	(32,5)	14,7	
	7	7	8,9	1,30	1,65	Dextranjern ≈ 150 mgFe Ferroaskorbat ≈ 45,5 mgFe	56,8	< 0,1	2,7	43,2	4,7	
				1. dag (1. day)	4. dag (4. day)							
	1	14	6,2	2,88	3,33	Ferroaskorbat ≈ 45,5 mgFe kontrol	73,1	< 0,1	1,1	26,9	38,1	
	8	14	5,3	2,84	3,18	Ferroaskorbat ≈ 45,5 mgFe kontrol	69,9	< 0,1	3,6	30,1	30,0	
	2	14	6,1	2,86	3,38	Ferroaskorbat ≈ 45,5 mgFe 1 g askorbinsyre dgl.	73,1	< 0,1	2,2	26,9	37,0	
	1	14	5,3	3,48	3,69	Ferroaskorbat ≈ 45,5 mgFe 0,5 g askorbnisyre dgl.	54,1	< 0,1	2,9	45,9	52,7	
	11	14	4,6	1,96	2,62	Ferroaskorbat ≈ 45,5 mgFe 0,5 g askorbinsyre dgl.	95,2	< 0,1	6,1	(4,8)	8,7	
				1. dag (1. day)	5. dag (5. day)							
	11	11	8,0	3,24	3,75	Ferroaskorbat ≈ 55,4 mgFe kontrol	85,1	< 0,1	0,0	14,9	7,4	
	8	11	7,6	3,42	3,75	Ferroaskorbat ≈ 55,4 mgFe 0,5 g askorbinsyre dgl.	77,5	0,1	0,2	22,4	11,2	
IV	12	11	6,9	3,25	3,65	Ferroaskorbat ≈ 55,4 mgFe 0,5 g askorbinsyre dgl.	99,2	< 0,1	0,1	0,8	6,1	
	1	11	8,2	3,22	3,55	Fruktosejern ≈ 55,4 mgFe	82,1	0,1	5,0	(17,8)	3,1	
	3	11	7,0	3,45	3,80	Fruktosejern ≈ 55,4 mgFe	78,8	< 0,1	1,4	21,2	14,3	$\pm$ 0,70 %
				1. dag (1. day)	7. dag (7. day)							
	1	7	9,5	2,05	2,83	Ferriammoniumcitrat ≈ 44,2 mgFe	86,6	0,7	0,1	12,7	1,9	
	8	7	7,1	1,78	2,34	Ferriammoniumcitrat ≈ 44,2 mgFe	91,9	< 0,1	0,1	8,1	3,2	
	5	10	11,4	2,26	3,04	Ferriammoniumcitrat ≈ 44,2 mgFe	91,1	< 0,1	0,2	8,9	1,5	
V	6	10	14,8	2,26	3,33	Ferriammoniumcitrat ≈ 44,2 mgFe	96,4	< 0,1	0,2	3,6	0,9	
	4	13	9,2	2,53	3,47	Ferriammoniumcitrat ≈ 44,2 mgFe	101,2	0,2	0,2	0,0	2,3	
	11	13	8,7	2,20	3,14	Ferriammoniumcitrat ≈ 44,2 mgFe	94,2	0,1	0,2	5,7	1,8	
	9	17	11,4	2,49	3,30	Ferriammoniumcitrat ≈ 44,2 mgFe	96,0	0,1	0,2	3,9	4,1	$\pm$ 0,90 %

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Meat Diets: Effect of Supplements of Calcium and Ferrous Carbonates on Rats Fed Meat

T. MOORE, I. M. SHARMAN, B. J. CONSTABLE, K. R. SYMONDS, P. E. N. MARTIN AND EDNA COLLINSON

Dunn Nutritional Laboratory, University of Cambridge, England, and Medical Research Council

Research on metabolic interrelationships between calcium and iron commenced at least 30 years ago. Two main lines of advance may be discerned. First, investigations were made on the influence of calcium salts on iron absorption and hematopoiesis. The second topic for study was the interference in bone formation by high intakes of iron salts.

Early work by von Wendt ('05) and Sherman ('07) suggested that calcium assists the absorption of iron. In agreement with this conclusion Orten et al. ('36) found that low hemoglobin levels, in rats given a diet deficient in minerals, could be corrected by supplements either of iron or of calcium. Similar observations were made by Day and Stein ('38) who suggested that the role of calcium in hematopoiesis is not primary, but that it acts by opposing an excess of phosphorus. Vitamin D also had a beneficial effect on hematopoiesis, presumably by increasing the absorption of calcium.

Observations by other workers, however, were different. Shelling and Josephs ('34) reported that in rats iron retention and hemoglobin formation were inversely related to the Ca:P ratio in the diet. Kletzien ('35, '38, '40) also opposed the view that calcium assists in iron metabolism. After making rats anemic by feeding them dried milk, he transferred them to a diet containing fixed amounts of iron, but with different amounts of calcium. Iron storage, particularly during the early stages of recovery, was inversely related to the calcium intake. Richards and Greig ('52) observed ill effects on reproduction and hematopoiesis when excessive amounts of calcium carbonate were added to the diet of breeding mice and their young. The anemia produced in these animals was investigated by Grieg ('52) who found hematological changes typical of iron deficiency. These changes could be prevented, without removing the calcium, by the addition of iron to the diet. Chapman and Campbell ('57a, b, c) followed the procedure of Kletzien in first feeding their rats milk powder. When anemic, the rats were transferred to diets consisting mainly of flour, fortified with graded amounts of various calcium and iron salts, and used for studies of hemaglobin regeneration and iron storage. When the intake of iron was low it was found that an excess of calcium interfered with the metabolism of iron.

Evidence on our second topic, showing that an excess of iron may interfere with the metabolism of calcium, was reported by Waltner ('27). Rickets was produced in young rats by feeding them a stock diet, to which 2% of reduced iron was added. Cox et al. ('31) found that the addition of ferric or aluminum salts to the diets of guinea pigs or rabbits caused marked decreases in bone ash and in blood phosphorus. It appeared, as in the work of Waltner, that bone formation was impaired by interference with the absorption of phosphorus. Brock and Diamond ('34) and Deobald and Elvehjem ('35) produced rickets, in rats and chicks, respectively, by adding iron salts to diets which were otherwise adequate for bone formation. Rehm and Winters ('40) found that the addition of ferric chloride to the diets of rats, given controlled amounts of food, caused reductions in the calcium and phosphorus content of their bones.

The present work was planned as an extension of experiments on the severe calcium deficiency which can be produced

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in rats by their restriction to a diet of raw meat (Scott and Scott, '60; Moore and Sharman, '60). In agreement with studies by Greaves et al. ('59) on cats, it was found that young rats, after receiving meat for 6 to 7 weeks, developed severe softening of the bones, which often resulted in fractures. Many of these animals were anemic, which suggested that it might be worthwhile to try the effect of liberal supplements of calcium and of iron, either separately or combined. In animals with and without supplements, we have studied growth, bone formation and hematopoiesis. Dental pigmentation (Ratner, '35–'36) and histological staining of organs have been used as additional indications of the iron status. Our results, obtained with the use of a basic diet which differed greatly from those used in previous work, provide further evidence of metabolic interplay between calcium and iron.

MATERIALS AND METHODS

Rats. Purebred, piebald, male rats were used. They were reared with a stock diet until they reached body weights of about 75 gm, and were then transferred to their experimental diets.

Diet. Minced beef steak, if not in a sufficiently fine state, was reminced, to prevent the rats from picking out fat from lean meat. The average fat percentage, estimated by Soxhlet extractions with ethanol followed by ether, was 16.8. Calcium and iron, estimated by methods of the AOAC ('60) averaged 17 mg and 4.9 mg/100 gm of wet weight, respectively. According to food tables (McCance and Widdowson, '60) raw steak contains 276 mg of phosphorus/100 gm. Adequate doses of vitamins A and D were given.

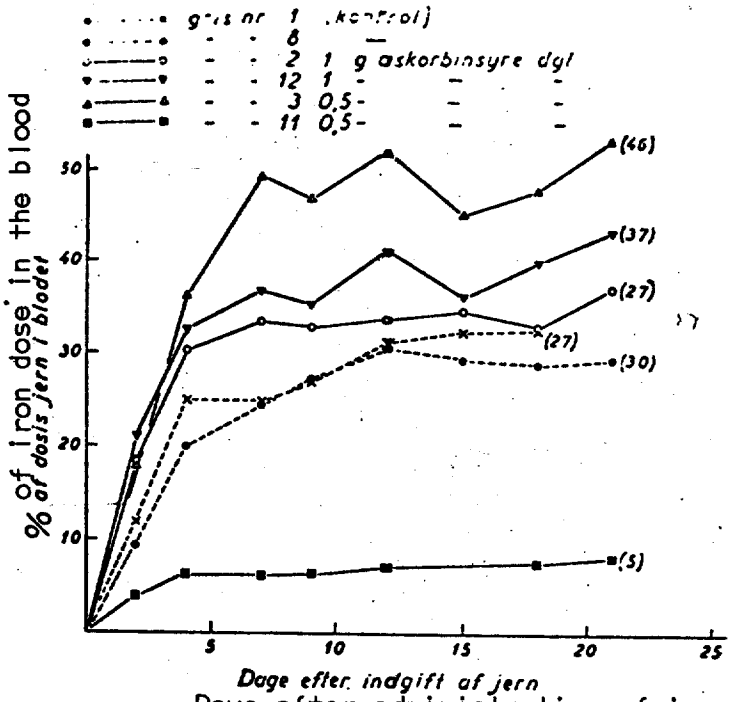
Mineral supplements and grouping of rats. Six groups of rats received meat, with supplements as follows.

Group	Calcium carbonate	Ferrous carbonate
	%	%
1	0	0
2	0.5	0
3	5.0	0
4	0	2.5
5	0.5	2.5
6	5.0	2.5

Group 4 contained 8 rats, but all the other groups only 5 rats. For convenience the experiment was carried out in two instalments. The first instalment included three animals each in groups 1, 2, 3 and 5, and the second part the remaining animals. The ferrous carbonate contained an equal quantity of glucose, which was put in by the makers as a stabilizing agent. Compensation for this small amount of carbohydrate in the diets not containing $FeCO_3$ was not considered to be worth the extra labor that it would have involved.

Most of the rats were killed after they had received their meat diets for 63 to 67 days. In the second instalment of the experiments, however, those rats receiving no added calcium not only stopped growing, but became so weak that it seemed unlikely that they would survive for the full experimental period. They were therefore killed after 42 to 50 days. Since the rats that were killed early had all stopped growing, it is unlikely that survival for the whole experimental period, even if it had been possible, would have brought their body weights and bone measurements any closer to those observed in the groups given calcium.

Before killing the rats were anesthetized with ether, and then exsanguinated by withdrawal of blood from the inferior vena cava. Red cell counts, packed cell volume and hemoglobin were measured by routine methods. Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentrations were calculated from these results. Radiographs were taken of many of the rats. From each animal a femur was dissected out. It was carefully freed from muscle and sinew, measured for length, and weighed. The bone was then boiled repeatedly with a mixture of ether and ethanol, to remove fat, and was broken at about the middle of the shaft. Measurements of the thickness of the bone wall were made on fragments, so obtained, by means of a micrometer. The pieces of bone were then collected together for ashing, and the ashes were subsequently weighed. The ashes of each group were then pooled for mineral analyses. Calcium and iron were again estimated by A.O.A.C. methods.



Figur 1. Askorbinsyrens indflydelse på jernresorption (tallene i parentes) og på inkorporationen af jern i hæmoglobin.
(Influence of ascorbic acid on absorption of iron (figures in brackets) and on incorporation of iron into hemoglobin).

Paraffin sections of liver, spleen, and sometimes other organs were prepared, and stained with hematoxylin and eosin, or with potassium ferrocyanide and eosin. Calcium and iron were estimated in the livers, pooled in groups, by the methods already mentioned. As a possible indication of the adequacy of the iron intake, observations were made on the teeth of the rats. The incisors were examined frequently during life for the degree of normal brown pigmentation seen on their anterior surfaces, and after death the upper incisors were extracted. After these had been washed, and graded according to their degree of pigmentation, they were ignited, so as to detach the enamel from the dentin. The enamel was then ashed for iron analyses. The hair on the head and shoulders of the rats was examined for loss of its normal black, or dark brown pigmentation. Occasionally feces were collected, for weighing and superficial inspection.

OBSERVATIONS

Growth and body composition. The initial, maximal and final mean body weights of groups are given in table 1. Growth was slow, and ceased altogether after about 30 days, in those animals that received no calcium carbonate. In contrast, the animals that received either 0.5 or 5.0% of calcium carbonate continued to grow throughout the period of the experiment, attaining body weights that were usually at least twice those of rats that received no calcium. Comparisons of the influence of the two levels of calcium carbonate on growth rate were made by combining the data for groups 2 and 5 and groups 3 and 6. The mean weight increase of 186 gm with 5.0% of calcium carbonate was significantly lower than the mean of 239 gm with only 0.5% of calcium carbonate ($P < 0.01$).

The effects of the ferrous carbonate on growth varied according to whether calcium carbonate was allowed or withheld. When no calcium carbonate was given the rate of weight increase was further reduced by the inclusion of ferrous carbonate in the diet. Thus in group 4, given iron, the mean difference between the initial and final body weights was only 62 gm as compared with 90 gm in group 1, with no iron ($0.01 < P < 0.05$).[1] In the groups given either 0.5 or 5% of calcium carbonate, weight increases were somewhat greater when the diet contained ferrous carbonate than when it was omitted, but the differences were not statistically significant.

The low weights of the rats not given calcium were due not only to the small size of the skeletons but to the poor development of their muscles, and the virtual absence of body fat. In contrast, their visceral organs were only slightly smaller than those of the control animals. In the groups not given calcium carbonate (1 and 4), the livers averaged 7.0 and 6.1% of the body weight, as compared with 4.2% for the combined rats in all the groups which received calcium. This disparity slightly exceeded the variation with body weight to be expected from the work of Webster et al. ('47). Thus, according to these workers the livers of male rats weighing 125 gm, typical of groups 1 and 4, should have represented 5.7% of the body weight, as compared with about 4.0% in rats weighing 275 gm, typical of the rats in the groups given calcium carbonate.

The testes also represented a higher percentage of the body weight in those rats deficient in calcium than in those given supplements (table 1). Thus these organs averaged 1.62 and 1.97% of the body weight in groups 1 and 4, respectively, as compared with 1.02 for the combined rats of the groups given calcium carbonate. These observations may be compared with percentages of 1.0 and 0.92 found by Ahmed[2] for the testes of normal piebald rats averaging 129 and 281 gm in body weight, respectively.

Further, minor differences were found according to whether the rats received the adequate or the excessive level of calcium carbonate. Adequate calcium was associated with the higher liver values and excess of calcium with the higher values for testes. Thus the liver represented a higher percentage of the body weight in group 2

[1] Subsequent experiments have confirmed this effect of excess of ferrous carbonate in retarding growth in rats that are deficient in calcium.

[2] Ahmed, S. I. 1957 Reproductive organs of the male rat in deficiencies of vitamins E and A. Thesis, Cambridge University Library, p. 28.

than in group 3 (not significant) and in group 5 than in group 6 ($0.05 < P < 0.1$). For the testes the relationship was reversed, the percentage being greater in group 3 than in group 2 ($0.05 < P < 0.1$) and in group 6 than in group 5 ($P < 0.01$).

Bone formation and calcification. The skeletons of all rats not given calcium carbonate (groups 1 and 4) were very soft and fragile, and offered little or no resistance to cutting with scissors. Many animals, when x-rayed, showed fractures of the leg bones and ribs. Great care was taken to avoid breakages during dissection.

In all rats given calcium carbonate the length and wet weight of the femur showed little variation (table 2). The femurs of the rats not given calcium carbonate, in accordance with the size of the animals, were much shorter and lighter than those of the rats allowed calcium. The differences in calcification were confirmed by measurements of the thickness of the bone wall. The average thickness of the bones from all the rats deficient in calcium (group 1 and 4) was only 0.25 mm, as compared with 0.66 mm in the rats receiving calcium carbonate (groups 2, 3, 5 and 6). The ash and calcium contents of the bones of the calcium-deficient rats were much lower than in those of the animals given calcium carbonate. Thus in the 4 groups given calcium the mean ash percentages of the wet bones fell within the range 30.5 to 33.0, and the calcium percentages within the range 10.4 to 12.8. For the groups not given calcium (1 and 4) the corresponding ash percentages were 11.5 and 6.7, and the calcium percentages 3.0 and 2.0.

Although the main factor determining adequate calcification was obviously the calcium content of the diet, the effect of the high iron intake on calcification was our particular interest. Tests of statistical significance were therefore made for the ash contents of the femurs between groups 1 (no Ca or Fe) and 4 (no Ca, 2.5% $FeCO_3$). For the total amount of ash per femur, which averaged 46 and 30 mg, respectively, the difference was significant ($P < 0.01$). For the percentages of ash in the wet bone the difference was also significant ($P < 0.001$).

Only traces of iron were found in any of the bones, with total amounts per femur of 4.1, 10, 13, 9.9, 18 and 8.9 µg in pooled bones from groups 1–6, respectively.

Hematopoiesis. Red blood cell formation was almost normal in group 2 (0.5% $CaCO_3$) even although no supplemental iron was given (table 3). In comparison with group 5 (0.5% $CaCO_3$ + 2.5% $FeCO_3$), however, the values in group 2 were significantly lower for hemoglobin ($0.01 < P < 0.05$) and packed cell volume ($0.01 < P < 0.05$). Much more severe anemia was found in groups 1 (no $CaCO_3$) and 3 (5% $CaCO_3$). Thus groups 1 and 3 were significantly lower than group 2 in hemoglobin ($0.01 < P < 0.05$ between group 2 and either group 1 or 3) and for packed cell volume ($P < 0.001$ between groups 2 and 1, and $0.01 < P < 0.05$ between groups 2 and 3).

All those groups given ferrous carbonate showed normal hematopoiesis, irrespective of the intake of calcium carbonate.

Dental pigmentation. In the groups not given ferrous carbonate (1, 2 and 3) the incisors of all the rats showed partial or complete depigmentation. Irregularities in the degree of pigmentation within the groups indicated the need for caution in reaching conclusions about the influence of the calcium intake. A rough system of scoring (table 4) however, allowed a comparison to be made between the groups; the average loss of normal depigmentation was much less in group 2 (0.5% $CaCO_3$) than in group 1 (no Ca) or 3 (5% $CaCO_3$).

All the rats given ferrous carbonate (groups 4, 5 and 6) had normally pigmented teeth, irrespective of the intake of calcium carbonate. The backs of the incisor teeth and the molars, however, remained unpigmented, which opposes the possibility that any contribution to the brown color of the teeth was caused by topical application of the iron salt in the diet.

Estimations of the minute amounts of iron in the enamel layers from pairs of incisors gave results that could not be correlated with the degree of pigmentation in individual rats. It appeared, therefore, either that iron was sometimes present in the enamel in a form not contribut-

TABLE 1

Mean weights of the body (initial, maximal and final) liver, and testes in groups of rats[1]

Group no.	Mean days fed diet	Body weight			Liver		Testes	
		Initial	Maximal	Final				
		gm	*gm*	*gm*	*gm*	%[1]	*gm*	%[1]
Meat diet with no iron								
1 (No $CaCO_3$)	55	75 ± 3.6[2]	165 ± 9.4	135 ± 4.5	9.4 ± 0.39	7.0 ± 0.24	2.2 ± 0.14	1.62 ± 0.08
2 (0.5% $CaCO_3$)	64	74 ± 3.5	301 ± 23.5	301 ± 23.5	13.2 ± 1.31	4.4 ± 0.25	2.9 ± 0.10	0.98 ± 0.07
3 (5% $CaCO_3$)	65	73 ± 2.7	254 ± 6.7	250 ± 8.0	9.9 ± 0.23	4.0 ± 0.16	2.9 ± 0.05	1.16 ± 0.05
Meat diet with 2.5% $FeCO_3$								
4 (No $CaCO_3$)	48	74 ± 1.1	136 ± 6.7	118 ± 8.6	7.1 ± 0.47	6.1 ± 0.13	2.2 ± 0.11	1.97 ± 0.06
5 (0.5% $CaCO_3$)	65	70 ± 2.7	322 ± 14.6	321 ± 14.6	13.9 ± 0.59	4.3 ± 0.18	2.8 ± 0.06	0.88 ± 0.03
6 (5% $CaCO_3$)	64	73 ± 0.5	268 ± 8.4	267 ± 9.2	10.3 ± 0.30	3.9 ± 0.06	2.8 ± 0.06	1.04 ± 0.02

[1] Calculated on the final body weight.

[2] Standard deviation.

TABLE 2

Mean femur length, wet weight, ash and calcium content for groups of rats

Group no.	Mean days fed diet	Final body wt	Femur					
			Length	Weight	Ash		Calcium	
		gm	*mm*	*mg*	*mg*	*%*[1]	*mg*	*%*[1]
Meat diet with no iron								
1 (No $CaCO_3$)	55	135± 4.5[2]	28.1±0.25	403± 2.3	46± 1.5	11.5±0.32	12	3.0
2 (0.5% $CaCO_3$)	64	301±23.5	33.4±0.29	795±40.0	255±22.1	31.9±1.48	83	10.4
3 (5% $CaCO_3$)	65	250± 8.0	32.5±0.37	797±10.6	263± 5.5	33.0±0.84	100	12.6
Meat with 2.5% $FeCO_3$								
4 (No $CaCO_3$)	48	118± 8.6	26.5±0.60	456±21.9	30± 2.3	6.7±0.43	9	2.0
5 (0.5% $CaCO_3$)	65	321±14.6	33.0±0.55	818±28.5	250±15.2	30.5±1.34	87	10.6
6 (5% $CaCO_3$)	64	267± 9.2	32.8±0.20	758±13.9	246± 5.4	32.6±0.50	88	11.6

[1] The percentages of ash and calcium are expressed on a wet-weight basis.
[2] Standard deviation.

TABLE 3

Hematological observations on rats fed meat, with or without supplements of calcium and ferrous carbonates

Group	Mean days fed diet	Red blood cells	Packed cell volume	Hemoglobin	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular hemoglobin concentration
		millions/mm³	%	%	μ^3	$\mu\mu g$	%
Meat diet with no iron							
1 (No $CaCO_3$)	55	7.3 ± 0.98[1]	27.4 ± 1.95	9.3 ± 0.87	39.9 ± 4.44	13.4 ± 1.52	34.1 ± 2.77
2 (0.5% $CaCO_3$)	64	9.0 ± 0.52	43.8 ± 1.93	13.0 ± 1.04	48.9 ± 2.00	14.4 ± 0.77	29.5 ± 1.13
3 (5% $CaCO_3$)	65	8.5 ± 0.79	33.6 ± 2.65	9.2 ± 0.98	39.9 ± 1.54	10.8 ± 0.36	27.1 ± 1.10
Meat diet with 2.5% $FeCO_3$							
4 (No $CaCO_3$)	48	7.8 ± 0.35	53.0 ± 1.40	16.9 ± 0.41	68.6 ± 1.68	21.9 ± 0.66	31.9 ± 0.29
5 (0.5% $CaCO_3$)	65	7.7 ± 0.63	50.8 ± 1.23	17.3 ± 0.99	66.8 ± 3.88	22.5 ± 0.82	33.9 ± 1.24
6 (5% $CaCO_3$)	64	7.3 ± 0.14	48.8 ± 1.78	15.7 ± 0.35	67.4 ± 1.05	21.7 ± 0.23	32.1 ± 0.65

[1] Standard deviation.

TABLE 4

Scoring for pigmentation of pairs of upper incisor teeth of rats fed meat, with or without supplements[1]

Group	Meat diet with no iron			Meat diet with 2.5% $FeCO_3$		
Rat no.	1 (No $CaCO_3$)	2 (0.5% $CaCO_3$)	3 (5% $CaCO_3$)	4 (No $CaCO_3$)	5 (0.5% $CaCO_3$)	6 (5% $CaCO_3$)
1	2	6	2	8	8	8
2	0	2	0	8	8	8
3	4	6	2	8	8	8
4	4	4	2	8	8	8
5	2	6	0	8	8	8
6				8		
7				8		
8				8		
Means	2.4	4.8	1.2	8	8	8

[1] For each tooth: white = 0, cream = 1, deep cream = 2, light brown = 3, brown = 4; maximum for pair of teeth = 8.

ing to the pigmentation, or that the method of analysis was inadequate for the purpose required. Although the results seemed inconsistant for individual rats, however, a clear difference was found between those rats that received iron carbonate and those that did not. Thus for the combined rats of groups 1, 2 and 3 (no Fe) the iron content. per pair of incisors, was 16 to 40, mean 29 µg. For groups 4, 5 and 6 (2.5% $FeCO_3$) the range was 41 to 90, mean 67 µg. This difference between the rats receiving and not receiving ferrous carbonate was significant ($P < 0.001$).

Hypochromotrichia. In most of the rats not given iron carbonate. the hair on the head and shoulders, which is normally black, became grey. In contrast the hair in the same areas of all the rats given ferrous carbonate remained normally pigmented.

Deposition of iron in spleen and liver. The results of our histological studies are summarized in table 5. No iron could be demonstrated by ferrocyanide staining in any of the organs of the rats not given ferrous carbonate (groups 1, 2 and 3). All rats given ferrous carbonate (groups 4, 5 and 6) showed iron staining in the spleen. In sections stained with hematoxylin and eosin, light brown granules or concretions, presumably of haemosiderin, were observed in the vicinity of the reticuloendothelial cells of the red pulp. Ferrocyanide produced blue staining in the same regions, with the lymphatic areas free from staining. The intensity of staining in the spleen was not influenced by the calcium intake.

In the livers of the rats given ferrous carbonate, however, the deposition of iron was profoundly influenced by the calcium intake (fig. 1). The livers of all the rats of group 4 (Fe but no Ca)stained intensely with ferrocyanide. The staining was concentrated in the portal areas, and the cells surrounding the central veins were free from staining. Staining was most dense near the outer layers of the portal blood

TABLE 5

Iron staining of individual spleens and livers, and iron content of pooled livers of groups of rats fed meat with or without supplements

Group	Spleen staining[1]	Liver	
		Staining[1]	Fe
			mg./100 gm
Meat with no iron			
1 (No $CaCO_3$)	0	0	51
2 (0.5% $CaCO_3$)	0	0	24
3 (5% $CaCO_3$)	0	0	9
Meat with 2.5% $FeCO_3$			
4 (No $CaCO_3$)	+	++	415
5 (0.5% $CaCO_3$)	+	+[2]	73
6 (5% $CaCO_3$)	+	0	32

[1] Except when otherwise stated similar intensity of staining was observed in all members of group.
[2] In one out of 5 rats the liver did not stain.

Fig. 1 Iron deposition in the livers of rats fed meat, supplemented with 2.5% of ferrous carbonate, as demonstrated histologically. Paraffin sections stained with ferrocyanide and eosin, low magnification. (1) Typical of all the rats in group 4 (2.5% $FeCO_3$ only); intense iron staining in the portal areas. (2) Typical of 4 out of 5 rats in group 5 (2.5% $FeCO_3$ + 0.5% $CaCO_3$); moderate staining. (3) Typical of all rats in group 6 (2.5% $FeCO_3$ + 5.0% $CaCO_3$); no staining.

vessels, and diminished in intensity according to the distance of the liver cells from the portal areas. In group 5 (Fe with 0.5 $CaCO_3$) similar staining was seen in 4 out of the 5 rats, but was much less intense. In group 6 (Fe and 5.0% $CaCO_3$) the livers, in contrast with the spleens, were not stained by ferrocyanide.

Iron estimations confirmed the histological finding that calcium opposed the deposition of iron. This effect was seen even in those groups not given ferrous carbonate. The range of iron content varied from only 9 mg 100 gm of pooled liver in group 3 (no Fe and 5.0% $CaCO_3$) up to 415 mg 100 gm in group 4 (Fe but no Ca).

The testes of many of the rats given iron showed ferrocyanide staining, confined to a limited number of the interstitial cells.

Feces. As expected, the amounts and appearance of the feces varied widely according to the mineral content of the diet. Thus exploratory collections from one of the rats in group 1 (no Ca or Fe) gave only about 0.25 gm of small, dark brown pellets daily. A rat in group 2 (0.5% $CaCO_3$ but no Fe) excreted about 0.8 gm of feces daily, with pellets that were either dark brown or cream colored, according to the increasing age of the rat. In group 3 (5% $CaCO_3$ but no Fe) the daily excretion was nearly 3.0 gm, and the pellets were large, and almost white. In group 6 (5% $CaCO_3$ + 2.5% $FeCO_3$) the excretion, in the last few days of the experiment reached 6.0 gm daily, and the pellets were very large and colored light brown. Clearly the necessity to excrete large amounts of superfluous minerals did not prevent the rats from maintaining good health and growth.

DISCUSSION

Our observations confirm the conclusion that raw, boneless meat is inadequate as a source of calcium for young, rapidly growing rats. Calculations show that the amounts of calcium usually available in such meats could not supply the needs of the growing skeleton, even if absorbed and transported to the bones without wastage. Besides the absolute shortage of calcium in meat, however, the very low ratio of calcium to phosphorus may adversely affect absorption of calcium, and other metals. The influence of fat associated with lean meat in decreasing the amount of lean meat eaten, and hence making the calcium intake even more inadequate, has already been discussed (Moore and Sharman, '60). As part of the present work we have compared body weights of our rats with the weight of their organs, as typified by the liver and testes. The effect of the meat diet, without added calcium, was to

interfere with the development of the skeleton and musculature. Fat formation was even more severely affected, presumably as a secondary effect, since deposits were virtually absent. In contrast, the liver and testes were only slightly smaller than those of normal animals, and therefore made up higher percentages of the body weight. The conclusion that deficiency in calcium was the cause of these abnormalities was made clear by the normal development of the skeleton, and by the normal amounts of muscle and fat, in the rats given meat with supplements of calcium.

In addition to causing defective calcification, a diet of meat, without supplements, failed to maintain normal hematopoiesis. By merely feeding young, growing rats upon meat, without supplements, we readily produced anemia, without having to superimpose the nondietary stresses, such as bleeding or reproduction, found necessary for this purpose by some previous workers. This anemia occurred even though the total iron content of the meat must greatly have exceed the minimal requirement of the rat. However, only about 10% of the iron of beef is present in an ionizable, and easily digestible, form (Shackleton and McCance, '36).

The influence of the calcium intake on iron metabolism. Evidence has long been conflicting about whether calcium assists, or inhibits, the metabolism of iron. Our results show clearly that both these assumptions may be correct, according to the experimental conditions chosen, and the aspects of iron metabolism studied.

Thus in rats given meat, without supplements of iron, hematopoiesis was significantly better when the diet contained 0.5% of calcium carbonate than when it contained zero or 5.0% of calcium carbonate. Either deficiency of calcium, or great excess, depressed hematopoiesis. Possibly an adequate intake of calcium may aid iron metabolism by counteracting excess of phosphate, but great excess of calcium may compete directly with iron in common pathways of absorption. Our results on dental pigmentation agreed with the concept of an optimal intake of calcium for the efficient metabolism of iron, since less depigmentation was observed when the diet contained 0.5% of $CaCO_3$ than when it contained zero or 5%. On the other hand, the storage of iron in the liver, measured chemically, was inversely related to the calcium intake, and did not show the optimum in the group given 0.5% of calcium carbonate.

In the rats given supplements of ferrous carbonate both hematopoiesis and dental pigmentation were normal, irrespective of the intake of calcium carbonate. In the group given no calcium the normality of the blood and teeth was in striking contrast with the general weakness and emaciation of the animals, and indicated clearly that poor condition was not in itself a cause of defective iron metabolism. Even in these animals given excess of iron, however, a reflection of the calcium intake was seen in amounts of iron deposited in the liver cells. Thus we have seen (table 5 and fig. 1) that in the livers of rats given excess of iron, ferrocyanide staining was intense, weak, or absent according to whether the calcium intake was deficient, adequate, or excessive. The amounts of iron, estimated chemically, ran parallel to the intensity of staining. In contrast to the livers, the spleens of all the groups given iron showed about equal densities of staining. This difference between the effects of calcium on the deposition of iron in the liver and spleens suggests that there must be some corresponding difference in the mechanism by which iron is absorbed by these two organs.

A possibility, which we are at present investigating (Moore, '62), is that our supply of ferrous carbonate may have derived at least part of its hematopoietic activity from copper present as an impurity. In favor of this view the ferrous carbonate prevented hypochromotrichia, which has been reported as an effect of copper deficiency in rats (Hundley, '50) but not as an effect of iron deficiency. As already mentioned, however, Grieg ('52) has reported that the anemia induced in mice by excessive intakes of calcium carbonate responds to moderate doses of iron, presumably in pure form.

The influence of excess of iron on calcium metabolism. Our results support the view that an excess of iron can ad-

versely affect calcification. This occurred only when no supplements of calcium carbonate were given. In these circumstances the excess of iron caused the bones to contain even less ash, and calcium, than would have been found in uncomplicated calcium deficiency. The excess of iron also had a further effect in lowering the growth rate, which was already severely limited by the inadequacy of calcium. The possibility that the high intake of ferrous carbonate was poisonous in itself, and not as a factor aggravating the shortage of calcium, was ruled out by the normality of both calcification and growth in those rats given excess of iron carbonate in conjunction with supplements of calcium carbonate.

Dental pigmentation. Although the brown pigment of the rat's incisors is an iron compound (Ratner, '35–'36; Lowater and Murray, '37; Dam and Granados, '45; Moore and Mitchell, '55) dental depigmentation has recently been of interest more as a sign of deficiencies of vitamins A or E rather than of an inadequate iron intake. Ratner found less iron in the teeth of anemic rats than in normal controls given a different type of diet. Moore ('50), however, failed to induce dental depigmentation in rats by the omission of iron from the salt mixture in their basal diet. In the present work we have seen that clear differences were found between the degree of dental pigmentation, and the iron contents of the enamel, according to whether the rats were given or denied supplements of ferrous carbonate. Again the possible interaction of copper must not be excluded.

Other interrelationships between metals. Finally we may emphasize that the interrelationship between calcium and iron, studied in our experiments, must not be regarded as a unique phenomenon, but merely as one of numerous possible instances of interaction between mineral nutrients. As a few examples of other relationships we may remember the familiar balance between calcium and phosphate, and interactions between molybdenum and copper (Ferguson et al., '43) copper and zinc (Smith and Larson, '46) zinc and cadmium (Parizek, '57) and arsenic and selenium (Moxon, '38).

SUMMARY

1. Young male rats were fed a diet of raw minced beef, containing 17% of fat and with adequate supplements of vitamins A and D. Growth ceased after a few weeks, with indications of severe calcium deficiency. The bones were undersized, had thin walls and a low ash content, and were often fractured. The skeletal muscles were underdeveloped and fat depots were virtually absent. The liver and testes, however, were only slightly below normal in size, and hence represented abnormally high percentages of the total body weight.

2. With either adequate (0.5%) or excessive (5.0%) amounts of calcium carbonate added to the meat the rats remained outwardly in good general health, grew well, and had normal bones and body conformation. Growth was slightly less rapid, however, with the excessive than with the adequate addition of calcium carbonate.

3. Rats given meat with either no calcium carbonate or an excess, without supplements of iron, became anemic. Another sign of defective iron metabolism was loss of pigment from the incisor teeth. In contrast, rats given the adequate allowance of calcium carbonate had almost normal blood, and less severe dental depigmentation.

4. Liberal additions of ferrous carbonate (2.5%)to the meat had no effect on the growth or skeletal development of the rats when the diet also contained calcium carbonate, at either the adequate or excessive level. When the diet contained no calcium carbonate, the growth rate, the maximal body weights attained, and the ash contents of the bones were further reduced by the addition to the meat of ferrous carbonate.

5. No signs of iron deficiency, whether looked for in the blood or teeth, were seen in those rats given ferrous carbonate, irrespective of the calcium intake. Even undersized and feeble rats, suffering from severe calcium deficiency, were not anemic, and had normally colored teeth, provided they were given ferrous carbonate.

6. In histological studies no stainable iron was found in either the spleens or livers of rats not given iron. In the rats

given ferrous carbonate, stainable iron was invariably found in the spleen, in about equal intensity in each group. In the liver, iron staining was inversely related to the calcium intake, being intense with no supplement of calcium carbonate, moderate with 0.5%, and absent with 5.0%. Chemical estimations of iron in the livers of the rats, indicated concentrations that were directly related to the iron intake and inversely related to the calcium intake.

7. These observations confirm and amplify earlier claims that the metabolism of calcium and iron are interrelated. This interrelationship, which is probably nonspecific, is presumably typical of many similar interrelationships in mineral metabolism.

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JOHN FITCH LANDON, M.D., Editor

ACUTE IRON POISONING*

REPORT OF A CASE AND REVIEW OF THE LITERATURE

JAMES W. MURPHY, M.D.
CHARLES NEUSTEIN, M.D.
A. C. HOFFMAN, M.D.
HAROLD V. WINTERS, M.D.
AND
ALBERT L. GASKINS, M.D.
Brooklyn.

Although in wide use as a hematinic, ferrous sulfate has been generally considered harmless by the oral route, except for occasionally producing nausea, abdominal pain, constipation and diarrhea[1, 2]. The amount of gastric irritation seems to depend on the solubility of the iron preparation, as does the utilization. Special coatings, designed to prevent solubility in the stomach, have been developed to lessen minor gastric irritation in the tablet form. Very little attention has been directed to the more severe toxic potentialities of ferrous sulfate.

Hurst[3] in Boston had a case of encephalopathy following large oral doses of iron and ammonium citrate (11 Grams per day for 3 weeks). Brock and Hunter[4] gave doses of 5-8 Grams per day and Blaud's pills (6 Grams a day) for 9 days, to children 7 to 14 years of age with no mention of toxicity. Reznikoff[5] gave a normal man iron and ammonium citrate in amounts equivalent to 2.0 Grams of metallic iron a day, without toxicity.

*From the Cumberland Hospital Pediatric Service of Dr. Thurman B. Givan, Brooklyn, N. Y.

In 1947, two fatal cases of ferrous sulfate poisoning were reported by Forbes[6], one in a three-year-old child, and the other in a one-year-old infant. They swallowed 50 tablets and between 30-35 tablets, respectively, of a preparation containing ferrous sulfate 0.2 Grams, copper sulfate 2.6 milligrams and manganese sulfate 2.6 milligrams per tablet. Both children developed signs and symptoms of gastro-intestinal irritation, restlessness, coma and shock. The first case was featured primarily by vomiting and icterus; the second by hematemesis. The older patient lived 53 hours and the younger patient 30 hours.

Thomson[7] reported a fatality with respiratory distress of a 16-month-old infant who ingested 26 tablets of a similar preparation of ferrous sulfate. He also records a survival in a 2-year-old child who swallowed 10 tablets of the same preparation, with occult blood detectable in the stool nine days later.

Also in 1947, Prain[8] reported a fatality in an 11-month-old infant who swallowed an unknown quantity of tablets containing iron, copper and manganese. She died 39 hours later, after a period of apparent well-being. Lindquist[9] reported a nonfatal case of ferric chloride poisoning in a 2½-year-old girl, with hematemesis, bloody stool and collapse. Smith, Jones and Cochran[10] report a single fatal case of iron poisoning. They suggest that the gray cyanosis noted in their case was probably due to methemoglobinemia because of definite improvement of the cyanosis after methylene blue therapy.

Thomson[11] reviewed six cases at the Dundee Royal Infirmary, including 2 fatalities previously recorded[7, 8], for the five-year-period from 1944 to 1949. The prominent findings in the four cases that recovered were pallor, drowsiness, vomiting and the passage of dark brown to black stools.

The following patient is of interest in that, unlike most previously reported cases, she demonstrated toxicity to a large amount of ferrous sulfate alone, surviving an amount bordering on the fatal dose[12].

CASE REPORT

A 30-month-old white female child was admitted to Cumberland Hospital on June 22, 1950, at 10:20 P.M., because of "vomiting and gagging".

One day prior to admission, the patient's mother had donated

blood and was given 100 tablets of ferrous sulfate (0.2 Grams per tablet) in an envelope. At 1 P.M. the day of admission the mother noticed the child sucking the tablets. The child was not near any window, sink, refuse-can or bathroom where she might have discarded some of the tablets. After a careful search only 25 tablets were recovered from the floor and envelope. At 5 P.M. (4 hours after ingestion) the child began to vomit blood-tinged material. The vomiting recurred four or five times and was associated with the passage of two to three diarrheal stools. The past history was non-contributory. The family history revealed that the mother had had infectious hepatitis during her sixth month of pregnancy.

On admission the physical examination was that of a well-developed, well-nourished, pale child who was playful, alert, interested in her surroundings, and who did not appear acutely ill. The pulse was 150, the temperature 99° F. (37.2° C.), respiration 16, blood pressure 96/50 and weight 11.7 Kg. The heart, lungs, abdomen, extremities and neurologic examinations were all normal. The only positive findings were a mild tonsillitis and pharyngitis.

The stomach was lavaged with 1,500 cc. of normal saline. The initial returns were grossly bloody but subsequent returns were clear. Vomiting occurred during the lavage, but no tablets were returned. The patient was treated with sodium bicarbonate, aluminum hydroxide gel, and penicillin in oil. After the first dose of sodium bicarbonate, the patient vomited approximately 100 cc: of pinkish fluid with particles of blood dispersed throughout the vomitus. There was no further vomiting during the patient's hospital stay. The temperature never rose higher than 99.8° F. (37.7° C.). The child had a fair appetite and took a normal diet after the first 24 hours during which time she was on a milk regime. Stools were normal after the passage of two tarry stools within the first 24 hours.

The child remained alert and playful throughout her period of hospitalization and showed no evidence of hepatic or renal injury. At no time did she appear very ill. The laboratory reported normal findings on repeated urine examinations, except for a 1 plus sugar in the first specimen. Complete blood counts on June 23 and 28, 1950 were reported as hemoglobins of 86 per cent and 78 per cent, red blood cells 4.3 million and 4.0 million, white blood cells 16,000 and 10,800. Blood differential smears showed

polymorphonuclear cells 63, lymphocytes 32, monocytes 5 and polymorphonuclear cells 50, lymphocytes 46, eosinophiles 4, respectively. Stool examination on June 23, 1950 was negative for occult blood. Kline test was negative. Phenolsulfonphthalein test on June 30, 1950 was negative. Liver function tests were reported as icterus index 5 units, Takata ara and cephalin flocculation negative. All were done on June 28, 1950. Roentgen examination on June 23, 1950 was reported as chest entirely normal, abdomen showed moderate gaseous distention of the stomach and a normal amount of gas throughout the intestinal tract, no evidence of tablets.

The patient made an uneventful recovery and was discharged on July 2, 1950, 11 days after admission.

DISCUSSION

The main features observed in this patient were pallor, hematemesis and tarry stool. This is in agreement with the findings reported by the British authors. Our patient presented no clinical or laboratory evidence of hepatic failure as was noted in the fatal cases reported by them.

On postmortem examinations of the few reported cases all showed edema and necrosis of the gastric mucosa, with congestion and hemorrhagic areas between the muscle layers. Prain[6] noted thrombosis of the submucosal veins under the necrotic areas of the gastric mucosa, with iron impregnated in the walls of the veins. Deeper veins that were not thrombosed showed thickened endothelium with masses of iron granules present. The changes in the liver varied from cloudy swelling to focal necrosis[6] with some iron demonstrable. The kidneys may show cloudy swelling and there may be necrosis of the malphighian corpuscles of the spleen.

The extent of the pathology found on the postmortem examinations seemed inadequate to explain the cause of death. Whether death is due to the absorbtion of iron into the general circulation or whether it is due to shock from tissue damage with absorbtion of the toxic products of necrosis has not been ascertained, although the extent of liver damage is usually not severe. Prain suggests that death follows collapse of liver function. He assumes that the damaged liver is capable of functioning well for some time, but cannot continue its detoxifying action in the presence of continued absorbtion of toxic products. The collapse of the liver permits the

distribution of toxic substances to the rest of the body, terminating in sudden death.

Following the reports of fatal cases from excessive ingestion of ferrous sulfate tablets by Forbes and Thomson[6, 7], Somers[12] investigated the relative oral toxicity of various therapeutic iron preparations. Using mice, rabbits and guinea pigs as subjects, it was found that ferrous sulfate, ferrous gluconate, and iron and ammonium citrate had the same toxicity. There was no difference in the toxicity of ferrous sulfate when copper and manganese were added to the preparation. Ferric iron preparations were found to be up to twice as toxic as ferrous preparations. No adequate explanation for this finding is presented in view of the fact that ferric iron is reduced in the stomach to the ferrous state. Blaud's pills were found to be one-fourth as toxic as the other preparations but also had a lower therapeutic value. This is apparently due to the relative insolubility of ferrous carbonate in gastric and intestinal contents. Clinically, the animals became prostrated within a few minutes after the oral administration of a toxic dose of iron. Reflexes became sluggish or disappeared, respiratory rate was increased, coma ensued, and death occurred within two to six hours. Postmortem examinations showed findings similar to those seen in humans, with no obvious cause of death.

The results obtained with Blaud's pills suggested the possible usefulness of sodium carbonate or bicarbonate as an antidote. Somers[12] therefore gave four rabbits known toxic doses of ferrous sulfate (3 Grams per Kilogram of body weight) and similar doses of sodium carbonate to two of the rabbits. One of these rabbits lived for two days and the other recovered completely. The two rabbits who did not receive sodium carbonate died overnight. The experiment was repeated twice with similar results.

Thomson[11] stresses the following features in the management of acute ferrous sulfate poisoning: (1) Emesis may be successful in ridding the body of swallowed tablets up to one hour after ingestion. (2) Gastric lavage should be performed with an aqueous bicarbonate solution to convert the corrosive ferrous sulfate to the much less irritant ferrous carbonate and to dilute the poisonous substance. (3) Bismuth preparations should be administered orally in order to protect the gastric mucosa. This measure is to prevent sudden fatal collapse possibly due to toxic absorbtion from damaged mucous membranes after a period of apparent improvement.

Roxburgh[13] reported a case of iron poisoning with recovery in a 16-month-old male infant. Dimercaprol (BAL) was administered daily for three days, but the author made no claim for its value. Treatment of iron poisoning with dimercaprol has been disappointing in animals[14]. The use of BAL seems to aggravate the effects of ferrous sulfate and ferric chloride, and a BAL-iron complex seems to be formed that is more toxic than the corresponding amount of iron salts alone.

Smith, Jones and Cochran[10] suggested that methemoglobin studies be done in these cases, and that they be treated with methylene blue if methemoglobinemia is found to be a factor.

SUMMARY

A case of ferrous sulfate toxicity in a child is reported and a brief review of the literature presented. Our patient developed marked gastric irritation with hematemesis following the ingestion of 15 Grams of ferrous sulfate (1.28 Gram per Kilogram). Unlike most of the previously reported cases there were no demonstrable systemic effects from ferrous sulfate alone. The potential danger of leaving iron preparations within the reach of children is illustrated. Possible methods of treatment are discussed.

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515 *East Third Street.*

REPORTS OF CASES

4,644 GRAMMES OF ORAL FERROUS SULPHATE (OVER 19 YEARS) WITHOUT APPARENT DAMAGE

KEVIN J. MURPHY, M.R.A.C.P.[1]

Princess Alexandra Hospital, Queensland

THE controversy regarding the fate of ingested iron remains unsettled, and the existence or otherwise of a mucosal barrier is still disputed. There have been four case reports (Case Records of the Massachusetts General Hospital, 1952, 1958, 1965; Turnberg, 1965) in which hæmochromatosis was attributed to, or associated with, prolonged ingestion of medicinal iron. In these cases, it appeared that excessive intake of iron caused excessive absorption. The following case report presents evidence in favour of a mucosal barrier for iron, as prolonged ingestion has not produced detectable damage.

CLINICAL RECORD

The patient was an unmarried hospital domestic, born in 1913. In 1948, she had been advised by one of the senior medical staff that she should take an iron tonic. Since then, she has insisted that she must have her iron, and has been given repeat prescriptions. The dose usually prescribed has been three tablets per day, each tablet containing ferrous sulphate (exsiccated) 200 mg., copper sulphate, 2·5 mg., and manganese sulphate, 2·5 mg. For approximately 12 months in 1956, she was given six tablets per day. In 1958, an effort was made to wean her from the tablets, but after a little over one month, she claimed that she was sick and could not manage without them. Her hæmoglobin value at that time was 14·9 grammes per 100 ml.

Her appetite has been normal throughout, and her diet has been adequate. Her weight has been stable. She abstains from alcohol. She has not had diarrhœa, chills, infection, kidney disease or jaundice. Her menstrual loss had been very light, and ceased 14 years ago at the age of 40 years. At no time has she had anæmia nor has she had detectable blood loss from the alimentary tract. She has never had glycosuria, and her postprandial blood sugar level has been normal. She has no abnormal skin pigmentation, and her liver and spleen are not clinically enlarged. Liver function tests have given normal results (including a bromsulphthalein retention of 2% after 45 minutes). Diagnex blue (Azure A carbacrylic resin) test indicated normal gastric acidity, and *d*-xylose excretion was normal. In 1965, the patient's serum iron level was 127 μg. per 100 ml., with total iron-binding capacity of 448 μg. per 100 ml., and in 1966 her serum iron level was 70 μg. per 100 ml., with a total iron-binding capacity of 300 μg. per 100 ml. After oral administration of ^{59}Fe, 1·8% of the iron appeared in the red cells at 10 days. Liver biopsy has not been performed, because the patient insists that she is quite well as long as she is given her iron tablets. Random checks of fæces revealed dark colour, and the test of Afifi *et alii* (1966) to check for ingestion of iron has given a positive result.

[1] Medical Supervisor.

DISCUSSION

The patient's reaction when her tablets were ceased, and the positive result of the test for the presence of ingested iron in fæces, suggest that she has taken the tablets as stated. She has not had anæmia, evidence of blood loss, malabsorption, chronic infection, or renal disease. She does not have the "achylia gastrica" which has been found to diminish absorption of oral iron (Cook *et alii*, 1964) or the malnutrition (Kleckner *et alii*, 1955) and/or alcoholic habits (Charlton *et alii*, 1964) which have been considered to promote iron absorption.

Apart from the Bantu cases, most reported examples of secondary hæmochromatosis, whether attributed to orally or parenterally administered iron, have been associated with a variety of blood disorders (Kent and Popper, 1960), and the underlying disease has been thought to contribute to the abnormal iron storage. Mendel (1964), in reviewing iron-storage disease, found only two cases (Case Records of the Massachusetts General Hospital, 1952 and 1958) which he accepted as instances of hæmochromatosis due solely to prolonged consumption of medicinal iron. However, the first of these patients had an anæmia of undetermined cause. Turnberg (1965) has since reported the case of a woman, aged 60 years, who had taken 20 Blaud pills per day for 26 years, and who had a fully saturated iron-binding capacity of serum, and a liver biopsy report of hæmochromatosis. Also, in Case 56, 1965, of the Massachusetts General Hospital (1965), the pathologist's opinion was that the diagnosis was "idiopathic hæmochromatosis", but the patient had taken iron pills for 15 years, so MacDonald (1966) would not accept the term "idiopathic".

I have been unable to find any accounts of long-term iron therapy without damage.

Cappell *et alii* (1957) suggested that iron absorbed from the gut promotes the hepatic cirrhosis of hæmochromatosis, but iron transfused in the hæmoglobin molecule is non-injurious. According to this theory, if our patient had absorbed 1·8% of the medicinal iron as well as her dietary iron, she should have hæmochromatosis. The low serum iron value, and the low percentage saturation of the serum iron-binding capacity, make hæmochromatosis unlikely (Morgan and Carter, 1960); they also make siderosis improbable, but do not exclude it (Higginson *et alii*, 1957). The patient could have hepatic siderosis with up to 50 grammes of iron in the liver, without cirrhosis (Cappell *et alii*, 1957).

The degree of variation in the serum iron level is within the range of normality (Zilva and Patston, 1966).

The percentage of administered iron found in the red cells at 10 days is a low normal figure, higher than in most cases of transfusional siderosis (Bothwell *et alii*, 1953), but similar to that found in Turnberg's case of medicinal hæmochromatosis (1965).

SUMMARY

A case of a woman who has consistently taken tablets of iron, copper and manganese for 19 years is described. The patient has no clinical or biochemical evidence of hæmochromatosis, but siderosis has not been excluded.

The present state would be consistent with either an efficient mucosal block for iron, or storage of iron without tissue damage.

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(From the State Pharmaceutical Laboratory, the Pharmaceutical Institute and Södersjukhuset, Stockholm.)

Absorption Experiments with Different Preparations of Ferrous Tartrate and Ferrous Chloride.

by

Nils G. Nordenson, Håkan Rydin and Erik Sandell.

(Received May 31, 1949).

Serum iron determinations after administration of iron in single doses (HEILMEYER and PLÖTNER 1937; WALDENSTRÖM 1940; VAHLQUIST 1941, and others), as well as studies of the absorption of radioactive iron (HAHN, BALE, LAWRENCE, and WHIPPLE 1939, and others) have added to our knowledge of the absorption of iron from the intestinal tract. Administration of iron in single doses does not, however, permit of an exact evaluation of the amount absorbed, but only of a comparison between the degrees of absorption of different iron compounds in the same subject, or of an estimate of the absorption of a particular iron compound under different experimental conditions.

In experiments of this kind the form of preparation of the iron compound does not so far seem to have been sufficiently considered, and it does not appear clear from the therapeutic results whether it is of quantitative importance for iron absorption that a particular iron compound be administered in a certain form.

The object of the present work has been to study the absorption in human subjects of different preparations of ferrous tartrate and ferrous chloride, these being the compounds commonly used in the Scandinavian countries. The principle of the investigations has been that of following the rise in serum iron concentration after administering a single dose of the preparation.

It is of the greatest importance for the absorption of iron from

the intestinal tract that disintegration of the preparation administered takes place in the manner most fit for the purpose. It is therefore necessary to give particular attention to the composition and technical production of the preparation.

Ferrous tartrate is a complex iron salt introduced into therapy by GJALDBÆK (1933). It is almost insoluble in water, but is stated to be absorbed without difficulty. Its particular suitability is probably due to the circumstance that its taste is only slightly metallic and that it is fairly resistant to oxidation by atmospheric air. Ferrous tartrate was at first given in uncoated tablets, but is now administered as tablets with a sugar coating, which eliminates the somewhat disagreeable taste. In addition it is thereby protected against oxidation during any excessively long storing. The present absorption experiments were made with ferrous tartrate tablets of 0.25 g each with and without sugar coating.

Preparations containing *ferrous chloride* have of late years been increasingly used. This compound should be particularly absorbable owing to its solubility in water. Furthermore, it is stated to be soluble in lipids. Its excellent effect in iron deficiency states has been demonstrated. Unfortunately, however, the preparation has a pronounced metallic taste, and it may give dyspeptic symptoms when ingested in large doses. Moreover, it is sensitive to atmospheric moisture and liable to oxidation. Ferrous chloride is administered in the form of sugar-coated tablets, pills or syrup. For the present experiments we used ferrous chloride syrup and sugar-coated tablets.

When a preparation is to be administered in the form of tablets with or without sugar coating, these must be so made as to secure a sufficiently rapid disintegration in the intestinal tract. Pharmaceutical literature contains numerous publications dealing with this important question of disintegration time *in vitro* and *in vivo*. THORMANN (1943) recently studied this problem.

We employed for our investigations a convenient method giving reproducible results, though the conditions of disintegration were not identical with those in the organism.

This method, recommended by various investigators, was first suggested by H. TRUNKEL (1931). In our modification the tablet is laid on a piece of wire gauze spread over a beaker, which is placed in a water bath containing just sufficient water for the gauge to be about 1 cm below the surface. The water is kept at a temperature of about 37° C. The saturated solution developing round the tablet will sink to the bottom and be replaced by fresh water. The time is determined for a tablet with

or without sugar coating to dissolve, disintegrate or soften so much that light pressure will make it fall apart without leaving a solid nucleus.

1. Procedure.

The experiments were made in Stockholm on female patients with normal blood values. None of the patients were suffering from a disease that might possibly influence the results of the experiments (neurosis, varicose ulcers, arterial hypertension, disseminated sclerosis, compensated heart diseases, asthma, etc.).

At 7 o'clock a.m. the fasting patients were given the iron preparation and a glass of water, immediately after a blood sample had been taken for determination of the normal serum iron concentration. Blood samples were then taken after 1, 2, 3 and 5 hours and in certain cases also after 6½ hours.

One week later a test was made on the same subject with the iron compound prepared in the other form.

Orientating experiments showed that some of the experimental subjects developed dyspeptic symptoms during the fasting period. All the patients were therefore given an evening breakfast at 9.30 p.m., and after that no food till 12.30.

Serum iron determination. The analyses were made by Agner's (1947) method, which is based on previous work by Heilmeyer and Plötner (1937), Barkan and Walker (1940) and Vahlquist (1941). Sodium pyrosulphite was used as a reducing agent. The method involves colorimetric determinations of the coloured complex of ferrous ions and o-phenanthroline.

Duplicate determinations from the same subject never differed by more than 4 %.

Both the ferrous tartrate tablets, with and without sugar coating, and the ferrous chloride syrup were kindly placed at our disposal by the dispensary *Vita Bjornen*, Stockholm. The sugar coated ferrous chloride tablets ("Ferrofer forte"), produced by the *Astra* factory, were bought at the dispensary. All the preparations were checked for iron content, and the tablets, whether sugar-coated or not, were tested for time of disintegration, by the procedure described above.

2. Experiments with Ferrous Tartrate.

As already stated, our investigation of ferrous tartrate consisted in comparing tablets with and without sugar coating. The

coated tablets). Blood samples for serum iron determinations were taken as described above. After one week the experiments were repeated on the same subjects, but now with the other form of tablet. Half of the subjects had the non-coated tablets first, and the other half the coated ones.

The results are seen in Table 1. The figures in brackets give the increases of the serum iron over the initial values. It appears that there were considerable individual variations in initial values as well as in the individual responses to the iron administered. This agrees with previous observations by Waldenström (1940), Vahlquist (1941), and Powell (1944). The average initial value of serum iron was 91.9 ± 5.7 $\mu g\%$ with S. E. $\sigma = 43.5$. It appears from Powell's table (1944) that this average value corresponds very closely to those found by Heilmeyer and Plötner (88.5) and by Moore, Arrowsmith, Quilligan and Rhead (97.6), whereas Vahlquist and Powell found higher values (123 and 117 respectively). The differences may possibly be due to the use of different methods for the serum iron determinations.

The average increases in the serum iron values (in $\mu g\%$) at the different stages of the experimental period are shown in Table 2.

Table 2.

	1 hr.	2 hrs.	3 hrs.	4 hrs.
Uncoated tablets	45	55	54	40
Coated tablets	36	49	52	40

The results show that there is no significant difference in absorbability between coated and non-coated ferrous tartrate tablets. The average values and the standard deviation have been calculated from the differences between the increases in serum iron in each experimental subject after administration of coated and of non-coated tablets.

Table 1.

Ferrous Tartrate.

The values indicate $\mu g\%$ Fe; the figures in brackets indicate the increase above the initial value.

Dose: 4 tabl. cont. 0.25 g (0.22 g Fe^{2+}).

Dose: 4 coated tablets cont. 0.25 g (0.22 g Fe^{2+})

Patient No.	Preparation	Initial value	1 hr.	2 hrs.	3 hrs.	5 hrs.
1		124	148 (24)	152 (28)	116 (−8)	139 (15)
2		208	215 (7)	—	219 (11)	—
3		130	172 (42)	217 (87)	241 (111)	—
4		164	158 (−6)	165 (1)	144 (−20)	81 (−83
5		76	190 (174)	—	294 (278)	259 (243)
6		166	163 (−3)	173 (7)	148 (−18)	126 (−40
7	Uncoated	69	85 (16)	90 (21)	114 (45)	94 (25)
8	tabl.	87	199 (112)	242 (155)	232 (145)	212 (125)
9	first	93	115 (22)	122 (29)	118 (25)	—
10		101	99 (−2)	102 (1)	94 (−7)	98 (−3)
11		69	226 (157)	310 (241)	—	294 (225)
12		24	33 (9)	57 (33)	55 (31)	33 (9)
13		120	147 (27)	160 (40)	131 (11)	94 (−26
14		80	123 (43)	156 (76)	154 (74)	146 (66)
15		40	116 (76)	187 (147)	179 (139)	173 (133)
16		160	134 (−26)	131 (−29)	135 (−25)	109 (−51
17		73	112 (39)	118 (45)	109 (36)	73 (0)
18		46	68 (22)	112 (66)	110 (64)	70 (24)
19		109	119 (10)	—	260 (151)	239 (130)
20		115	124 (9)	128 (13)	122 (7)	106 (−9)
21	Uncoated	128	173 (45)	216 (88)	232 (104)	200 (72)
22	tabl.	55	94 (39)	93 (38)	82 (27)	68 (13)
23	last	87	114 (27)	129 (42)	—	99 (12)
24		78	93 (15)	94 (16)	97 (19)	84 (6)
25		70	125 (55)	135 (65)	126 (56)	99 (29)
26		117	161 (44)	181 (64)	180 (63)	158 (41)
27		79	105 (26)	117 (38)	118 (39)	135 (56)
28		155	245 (90)	260 (105)	256 (101)	—
29		34	55 (21)	49 (15)	37 (3)	32 (−2)

Patient No.	Preparation	Initial value	1 hr.	2 hrs.	3 hrs.	5 hrs.
1		128	145 (17)	156 (28)	170 (42)	156 (28)
2		82	137 (55)	—	216 (134)	—
3		84	142 (58)	195 (111)	197 (113)	—
4		257	307 (50)	283 (26)	316 (59)	324 (67)
5		26	41 (15)	—	209 (183)	234 (208)
6		138	136 (−2)	155 (17)	159 (21)	165 (27)
7	Coated	51	55 (4)	79 (28)	88 (37)	69 (18)
8	tablets	120	168 (48)	229 (109)	256 (136)	254 (134)
9	last	96	100 (4)	96 (0)	94 (−2)	—
10		66	65 (−1)	66 (0)	64 (−2)	60 (−6)
11		48	248 (200)	287 (239)	—	259 (211)
12		22	37 (15)	62 (40)	53 (31)	38 (16)
13		141	160 (19)	179 (38)	159 (18)	112 (−29)
14		92	72 (−20)	90 (−2)	98 (6)	91 (−1)
15		45	94 (49)	147 (102)	152 (107)	153 (108)
16		136	131 (−5)	115 (−21)	98 (−38)	94 (−42)
17		75	76 (1)	75 (0)	68 (−7)	56 (−19)
18		53	73 (20)	76 (23)	75 (22)	63 (10)
19		172	232 (60)	—	218 (46)	216 (44)
20		96	108 (12)	144 (48)	152 (56)	155 (59)
21	Coated	103	134 (31)	228 (125)	197 (94)	158 (55)
22	tablets	65	71 (6)	80 (15)	72 (7)	62 (−3)
23	first	47	79 (32)	111 (64)	—	98 (51)
24		87	179 (92)	169 (82)	178 (91)	78 (−9)
25		59	68 (9)	84 (25)	84 (25)	59 (0)
26		153	178 (25)	187 (34)	180 (27)	145 (−8)
27		63	87 (24)	115 (52)	139 (76)	133 (70)
28		145	236 (91)	233 (88)	221 (76)	—
29		38	43 (5)	45 (7)	97 (59)	52 (14)

tablets contained 0.25 g ferrous tartrate (corresponding to 55 mg Fe) and, as excipients, sugar, starch and talc. The sugar-coated tablets were prepared in the same manner, and then coated in the usual way, as recently described by KÄLLROT (1916).

The ferrous tartrate content of the preparations was controlled, and disintegration tests were made as indicated above. The non-coated tablets disintegrated after 10 to 20 seconds, whereas the sugar-coated ones required 8 minutes. 30 experimental subjects were given 1.00 g ferrous tartrate (4 coated or non-

Table 3.

The values indicate $\mu g\%$ Fe; the figures in brackets indicate the increase above the initial value.

Dose: 2 coated tablets Ferrofer forte (0.15 g Fe^{++}). *Ferrous Chloride.* Dose: 9.9 g ferrous chloride syrup (0.15 g Fe).

Patient No.	Preparation	Initial value	1 hr.	2 hrs.	3 hrs.	5 hrs.	6½ hrs.
30		119	160 (41)	223 (104)	208 (89)	202 (83)	190 (71)
31		62	82 (20)	93 (31)	100 (38)	73 (11)	53 (—9)
32		53	60 (7)	89 (36)	80 (27)	70 (17)	65 (12)
33		21	81 (60)	88 (67)	77 (56)	36 (15)	24 (3)
34		135	203 (68)	336 (201)	368 (233)	297 (162)	269 (134)
35		94	98 (4)	151 (57)	177 (83)	158 (64)	131 (37)
36	Coated	136	140 (4)	163 (27)	218 (82)	196 (60)	190 (54)
37	tablets	39	43 (4)	55 (16)	72 (33)	72 (33)	69 (30)
38	first	130	169 (39)	218 (88)	211 (81)	166 (36)	140 (10)
39		44	132 (88)	- -	207 (163)	293 (249)	293 (249)
40		142	144 (2)	170 (28)	178 (36)	159 (17)	110 (—32
41		158	164 (6)	-	170 (12)	177 (19)	139 (—19
42		107	99 (—8)	110 (3)	129 (22)	101 (—6)	99 (—8)
43		42	84 (42)	182 (40)	279 (237)	202 (160)	181 (139)
44		99	94 (—5)	132 (33)	120 (21)	119 (20)	109 (10)
45		99	123 (24)	177 (78)	205 (106)	163 (64)	146 (47)
46		184	212 (28)	234 (50)	243 (59)	- -	224 (40)
47		83	147 (64)	229 (146)	219 (136)	177 (94)	151 (68)
48		101	159 (58)	202 (101)	220 (119)	189 (88)	168 (67)
49		36	74 (38)	75 (39)	88 (52)	66 (30)	58 (22)
50		48	129 (81)	168 (120)	175 (127)	147 (99)	141 (93)
51	Coated	100	142 (42)	148 (48)	134 (34)	107 (7)	97 (—3)
52	tablets	95	92 (—3)	149 (54)	169 (74)	133 (38)	110 (15)
53	last	68	78 (10)	82 (14)	86 (18)	92 (24)	109 (41)
54		69	142 (73)	222 (153)	302 (233)	277 (208)	259 (190)
55		57	132 (75)	296 (239)	437 (380)	456 (399)	445 (388)
56		37	58 (21)	81 (44)	92 (55)	102 (65)	113 (76)
57		115	152 (37)	216 (101)	276 (161)	268 (153)	140 (25)
58		85	106 (21)	146 (61)	170 (85)	125 (40)	101 (16)
59		70	82 (12)	129 (59)	132 (62)	149 (79)	140 (70)

Patient No.	Preparation	Initial value	1 hr.	2 hrs.	3 hrs.	5 hrs.	6½ hrs.
30		81	193 (112)	179 (98)	171 (90)	149 (68)	112 (31)
31		40	80 (40)	84 (44)	67 (27)	41 (1)	27 (—13)
32		69	77 (8)	75 (6)	64 (—5)	60 (—9)	51 (—18)
33		51	303 (252)	308 (257)	302 (251)	272 (221)	248 (197)
34		129	410 (281)	398 (269)	382 (253)	340 (211)	280 (151)
35		117	184 (67)	185 (68)	197 (80)	185 (68)	182 (65)
36	Syrup	185	216 (31)	244 (59)	234 (49)	184 (—1)	163 (—22)
37	last	52	148 (96)	280 (228)	327 (275)	242 (190)	191 (139)
38		109	167 (58)	179 (70)	200 (91)	193 (84)	163 (54)
39		158	338 (180)		364 (206)	396 (238)	339 (181)
40		98	148 (50)	152 (54)	156 (58)	123 (25)	78 (—20)
41		152	208 (56)	-	209 (57)	203 (51)	173 (21)
42		72	95 (23)	107 (35)	122 (50)	88 (16)	83 (11)
43		31	99 (68)	169 (138)	164 (133)	123 (92)	86 (55)
44		135	175 (40)	201 (66)	217 (82)	206 (71)	167 (32)
45		68	258 (190)	304 (236)	326 (258)	310 (242)	322 (254)
46		118	208 (90)	212 (94)	143 (25)	-	199 (81)
47		76	268 (192)	316 (240)	278 (202)	241 (165)	223 (147)
48		109	191 (82)	218 (109)	222 (113)	191 (82)	158 (49)
49		61	212 (151)	218 (157)	200 (139)	176 (115)	139 (78)
50		53	410 (357)	359 (306)	273 (220)	216 (163)	194 (141)
51	Syrup	157	270 (113)	299 (142)	304 (147)	276 (119)	275 (118)
52	first	104	206 (102)	216 (112)	216 (112)	182 (78)	160 (56)
53		97	199 (102)	296 (199)	281 (184)	206 (109)	172 (75)
54		102	206 (104)	234 (132)	253 (151)	192 (90)	187 (85)
55		55	293 (238)	455 (400)	476 (421)	464 (409)	420 (365)
56		65	220 (155)	304 (239)	262 (197)	231 (166)	149 (84)
57		89	254 (165)	280 (191)	249 (160)	240 (151)	228 (139)
58		65	173 (108)	192 (127)	194 (129)	168 (102)	149 (84)
59		105	205 (100)	241 (136)	229 (124)	196 (91)	194 (89)

to 2 tablets or 9.9 g syrup, which were given with a light meal consisting of tea and biscuits. The serum iron concentrations were determined on blood taken at the above-mentioned intervals.

The results are seen in Table 3. This investigation likewise involved 30 experimental subjects.

The average increases in serum iron (in $\mu g\%$) are indicated in Table 4.

3. Experiments with Ferrous Chloride.

In these experiments ferrous chloride syrup was compared with sugar-coated tablets.

Ferrous chloride syrup is included in Ed. XI of the *Swedish Pharmacopoeia* (1946). It consists of a solution of ferrous chloride in a concentrated sugar solution, to which have been added small amounts of citric acid to protect the ferrous ion against oxidation. The preparation contains 1.5 % Fe.

The sugar-coated ferrous chloride tablets are produced by the *Astra* factory ("Ferrofer forte") and contain 75 mg Fe per tablet. It may in this connexion be mentioned that the sugar coating of the ferrous chloride tablets is beset with considerable technical difficulties owing to the great sensitivity of the contents to moisture. If protective measures are inadequate the coating will be liable to crack and moisture will be able to penetrate and cause disintegration of the tablet. The problem seems, however, now to have been solved in a reasonably satisfactory manner.

Tested by the above technique the "Ferrofer forte" tablets showed a disintegration time of 1¼ hours, though this does not mean that the iron may not be dissolved in a shorter time. If we allow a tablet to disintegrate in a small volume of fluid and determine the Fe^{++} concentration from successive samples, we find a continuous liberation of ferrous ions.

In the organism this will probably correspond with a continuous solution of the ferrous chloride within a period of from ½ to 2 hours after oral ingestion. There is thus a pronounced difference between the ferrous chloride tablets and the ferrous chloride syrup, from which the whole of the iron is set free immediately after intake.

The experiments were first made with ferrous chloride in amounts corresponding in iron content to the previously used doses of ferrous tartrate (3 sugar-coated tablets or 14.9 g syrup). Dyspeptic symptoms occurred so often, however, that the experiments had to be discontinued. The doses were then reduced

Table 4.

	1 hr.	2 hrs.	3 hrs.	5 hrs.	6½ hrs.
Syrup	120	150	140	118	90
Coated tablets.....	32	76	97	80	61

There is a pronounced difference between the absorptions of these two preparations. The serum iron values are considerably higher after administration of syrup than of the sugar-coated tablets, and these high values are already reached after about 1 hour, whereas the rise occurs at a far slower rate after tablets.

Table 5 shows the difference between the increases in serum iron after ferrous chloride syrup and sugar-coated ferrous chloride tablets.

Table 5.

1 hr.	2 hrs.	3 hrs.	5 hrs.	6½ hrs.
89 ± 12	74 ± 14	43 ± 14	37 ± 13	29 ± 14

The differences are large after 1 and 2 hours, and still significant after 5 hours. This is probably due to the fact that in ferrous chloride syrup all the iron is administered in a dissolved state, whereas the tablets must first disintegrate slowly in the intestinal tract.

4. Comparison between Ferrous Tartrate and Ferrous Chloride.

It appears from the above Tables (2 and 4) that a considerably higher rise in the serum iron concentration is obtained by using the slowly disintegrating sugar-coated ferrous chloride tablets than after administration of ferrous tartrate. Further, the amount of iron employed for the ferrous chloride experiments was only two-thirds of that used in the experiments with ferrous tartrate.

The results argue in favour of a deficient absorption of iron after intake of ferrous tartrate, a fact that may well be accounted

for by the low solubility of this compound. The objection may, however, be raised to this view that ferrous tartrate under normal conditions is probably exposed to the action of the acid gastric contents over a considerably longer time than in these experiments, a fact which may possibly influence both the solubility and the absorbability. Serum iron concentrations ought to be observed over a somewhat longer period to provide a basis for more complete comparison between the two iron salts.

Summary.

A comparison has been made, on the basis of single doses, between the absorption of iron from sugar-coated and un-coated ferrous tartrate tablets, and of absorption from ferrous chloride syrup and sugar-coated ferrous chloride tablets.

No difference was found between the absorption from sugar-coated and from non-coated tablets when the time of disintegration was short.

Administration of ferrous chloride syrup gave a rapid absorption of iron and high serum iron values, whereas sugar-coated ferrous chloride tablets gave a slower absorption and a smaller rise in serum iron. This is due to the slow disintegration characterizing these tablets.

A higher rise in the serum iron concentration is obtainable with ferrous chloride than with ferrous tartrate.

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Copper and Iron Levels in Organs and Tissues of Fattened Swine

by

Cand. biol. science T. Pyanovskaya, V. Ivshina, and A. Yarotskiy
Uzbek Scientific-research Institute of Animal Husbandry

We studied the effects of vegetable protein, hydrochloric acid and various dosages of iron and copper sulfate on the productivity of fattened swine, and at the same time, the level of deposited iron and copper in the organs and tissues.

The pigs were selected for resemblance to their breed, divided by age and weight into five groups, and fattened, from 70 days of age to the attainment by the animals of 100 kg of live weight.

The ration of group I consisted of 12.9% cottonseed oil meal, digestible protein – to the extent of the VIZh norm; in the remaining groups, cottonseed oil meal constituted 27.9% of the ration, but the level of protein was 15% higher than the norm. Besides cottonseed oil meal, the rations of all of the groups included barley and corn, wheat bran, and green alfalfa.

In addition to the basic ration, animals of groups III, IV, and V received daily the trace elements iron and copper sulfate: each pig in groups III and IV received 2 g of iron and 1 g of copper sulfate, and in group V– 0.2 mg iron and 0.02 mg copper sulfate per 1 kg of live weight. Besides this, for animals of group III, the meal was processed with 36% hydrochloric acid, based on the calculation of 22 ml per 1 kg of dry meal.[1]

After a three-month fattening period during which the pigs attained 100 kg live weight, one swine from each group was killed and 2 were castrated with a complete analysis of the presence of iron and copper in the liver, thyroid gland, spleen and flesh.

It was established that from 2 months 10 days to 4 months of age the greatest increases of weight (416, 537 g) were in the group III pigs, which received a large dose of iron and copper sulfate, and at the same time hydrochloric acid.

After 4 months of age the increase in weight of animals in group III no longer surpassed the weight increases in groups I and II, and after 5 months, the presence of hydrochloric acid in the ration of group III, and, at the same time, the presence of iron and copper sulfate in the rations of groups III and IV inhibits growth of the animals. Weight increases in these groups in the period from 5 to 7 months of age were less than in groups I and II. Correspondingly, consumption of fodder units, particularly protein, was higher.

During the entire period of fattening, weight increases of all groups were large; however, they were somewhat higher in groups I and III- 580-578 g- and lower in group IV- 554 g.

The consumption of fodder per 1 kg weight increase was greater in group IV- 3.87 k. ed. and 572 g of digestible protein, less in group I- 3.61 k. ed. and 432 g digestible protein. The consumption of fodder was identical in groups II, III, and V- 3.70 k. ed. and 553 g digestible protein.

The same conformity was observed in the thyroid gland. The gland was larger in those animals which received larger doses of iron sulfate; the liver, on the other hand, was largest in those animals which did not receive iron sulfate (groups I and II).

The data obtained made it possible to draw the conclusion that during fattening on vegetables in the period of intensive growth of pigs (from 2 to 4 months of age) it is necessary to increase the protein content by 15-20% in comparison with the VIZh norm, and to include in the ration, iron, copper and hydrochloric acid (following the dosage of group III). In the period of fattening of animals from 4-5 months of age, it is also necessary to increase the protein content in fodder to 15%.

In this manner, weight increases in swine of various ages occurs in direct relation to the quantity of protein and trace elements in the ration.

Copper and iron contents of the organs and tissues were determined, and at the same time, the relation of their intake into the organs with the fodder. In the animals of all groups, copper content was greater in the liver and flesh, somewhat less in the thyroid gland and spleen. In comparisons among the groups more sopper was contained in the liver, flesh and spleen of the swine of group IV, and in the thyroid gland of animals in groups III and IV, receiving a larger dose of copper sulfate. In the organs of animals of groups I and II, not receiving trace elements, and group V, receiving less of them, the organs contained less copper.

The data on copper contained in the liver of experimental groups I, II, III, and V agrees with the data of other studies, but for group IV there exists some disagreement with the literature (the authors of the article found that copper content in this group was greater- 0.35 mg %).

In the flesh of animals of the experimental and control groups it was found that copper content was 2-3 times greater in comparison with the data of other authors, but least of all was the content in the flesh of group III.

Iron dispersed in the organs and tissues of swine was somewhat different from copper. Its content was least of all in the flesh, and particularly little in animals receiving the largest

doses of iron sulfate (groups III and IV). In groups of animals with an increased protein level, but not receiving trace elements (groups II and V), the iron content in the flesh agreed with data in the literature, namely: 2.2-2.2 mg %.

Much iron was contained in the spleen of swine of all groups; however, in animals receiving iron sulfate (groups III, IV, and V), it was greatest (see the table).

The accumulation of copper occurs in a large degree in the flesh and liver; with an increase in intake, deposition of it also increases (group IV).

Iron is basically deposited in the spleen and thyroid gland.

(1) Органы	(2) Содержание Cu и Fe (в расчете на натуральную влажность) по группам, мг%									
	I		II		III		IV		V	
	(3) медь	(4) железо	(3) медь	(4) железо	(3) медь	(4) железо	(3) медь	(4) железо	(3) медь	(4) железо
(5) Печень	1,45	16,59	1,38	11,2	1,51	7,3	1,91	11,08	1,42	9,87
(6) Селезенка	0,29	19,08	0,34	18,92	0,38	23,84	0,49	25,1	0,40	22,27
(7) Щитовидная железа	0,58	3,33	0,36	3,93	0,72	5,3	0,65	6,05	0,41	2,05
(8) Мясо	0,98	1,81	0,86	1,90	0,71	1,64	1,07	1,51	0,87	2,66

Table 1.

1- Organs; 2- Cu and Fe content (by calculations of the natural moisture) by groups, mg %; 3- copper; 4- iron; 5- liver; 6- spleen; 7- thyroid gland; 8- flesh.

Note: Copper determined by the method of M. A. Rish, iron- by the classical method of L. M. Petrun'kin and others.

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THE GASTROINTESTINAL ABSORPTION OF ORAL IRON-DEXTRAN AND FERROUS SULFATE

By Patrick A. Ragen, M.D.

Leonard Walker, Ph.D.

Gerald D. Sparling, M.D.

and

Randolph P. Pillow, M.D.

(From the Mason Clinic, Seattle 1, Washington)

New oral iron preparations of varying compositions are constantly being made available for therapeutic use in iron deficiency anemias. Several of these new compounds contain iron in the form of iron-dextran complexes. The rate of gastrointestinal absorption of the iron in the iron-dextran complex is not known. The efficacy of any oral iron preparation will depend upon the rate of gastrointestinal absorption and on the total amount of iron in the preparation. The purpose of this study was to compare the rate of gastrointestinal absorption of an oral iron-dextran complex with the rate of gastrointestinal absorption of ferrous sulfate in the same subjects. Known amounts of the iron compounds labeled with radioactive iron, Fe_{59}, were given orally. The difference between the amount of iron given orally, and the iron recovered in the stools, as measured with a scintillation counter, was assumed to represent the amount of iron absorbed.

The technique utilized for measuring the gastrointestinal absorption of oral iron was similar to that described by Bonnet, Hagedorn and Owen[1] in which small doses of iron were used. With small doses, a larger percentage of oral iron is normally absorbed so that it is possible to detect decreased absorption.

Materials. Fe_{59} as ferrous sulfate solution was prepared so that one microcurie of Fe_{59} was present in 50 μg. of elemental iron. The specific activity of the Fe_{59} in the iron-dextran complex* was lower, so that 354 μg. of elemental iron was present for each microcurie of Fe_{59}. A minimum dose of one microcurie was necessary because of the limited sensitivity of the scintillation detector employed. Therefore, the amount of elemental iron in a single dose of iron-dextran was approximately seven times greater than that present in a single dose of the ferrous sulfate.

Methods. The same methods were utilized for measuring the absorption of both the ferrous sulfate and the iron-dextran complex. The iron solution was measured into a wax drinking cup, and the subjects, in a fasting state, drank the solution, rinsing the cup several times. All subsequent stools were individually collected until a single stool contained less than 1% of the administered dose, usually for 3 or 4 days. The subjects were initially given the ferrous sulfate solution. Two weeks later, which was at least a week after the stools no longer contained significant Fe_{59} from the initial dose, they received the iron-dextran complex.

The activity of the Fe_{59} in the stools was determined by measuring over a well counter. Pilot samples of both iron compounds were prepared containing the same amount of Fe_{59}, one microcurie, as was present in the oral doses. The activity found in the stools of each patient was compared with that of the pilot, that is, of the oral dose. The amount

*The organic iron-dextran complex, "Jefron," was obtained from the Pitman-Moore Company, Indianapolis, Indiana. It has a molecular weight of 30,000 and contains 45% iron by weight.

iron not present in the stools was assumed to have been absorbed.

Serum iron determination was performed according to the method of Fister[3].

Results. Eight adults, 7 women and one man, all apparently healthy, ingested the ferrous sulfate solution containing one microcurie of Fe_{59} with 50 μg. of elemental iron. Two weeks later, all these subjects, plus one other healthy adult woman, ingested the iron-dextran complex which contained one microcurie of Fe_{59} and 354 μg. of elemental iron. The hemoglobin of the 8 women ranged from 11.6 to 13.5 gm. per 100 ml. of blood. Their serum iron ranged from 50 to 110 μg. per 100 ml. The one man had a hemoglobin of 15 gm. per 100 ml. of blood, and a serum iron of 95 μg. per 100 ml.

As shown in Table 1, the range of absorption of the ferrous sulfate was 2% to 88%, with an average of 52%. The absorption of iron in the form of iron-dextran complex ranged from 37% to 78% of the oral dose with an average of 51%.

Four subjects absorbed 7 to 28% (average 16%) more of the oral iron from ferrous sulfate than from iron-dextran complex. The other 4 subjects absorbed from 6 to 20% (average 14%) more of the oral iron when given the iron-dextran complex. However, the absolute amount of iron was 50 μg. in the ferrous sulfate doses, and 354 μg. in the iron-dextran doses. Therefore, the absolute amount of elemental iron absorbed in the ferrous sulfate studies ranged from 14 μg. to 44 μg., with an average of 26 μg., as compared to a range of 131 μg. to 278 μg., with an average of 180 μg. for the iron-dextran complex.

Discussion. The fractional gastrointestinal absorption of oral ferrous sulfate and iron-dextran was essentially the same. The iron-dextran complex contained approximately seven times as much elemental iron as the ferrous sulfate solution. It has been well-documented that the percentage of oral iron absorbed decreases with increasing doses (Bonnet, Hagedorn and Owen[1], Bothwell, Pirzio-Biroli and Finch[2]). Therefore, the amount of iron absorbed from the iron-dextran complex would exceed that from ferrous sulfate, if similar doses are used.

Therapeutic effectiveness of the oral iron-dextran compound was not evaluated. This could be measured clinically by treating patients with iron deficiency

TABLE 1.—GASTROINTESTINAL ABSORPTION OF ORAL IRON

	Ferrous Sulfate Absorption (50 μg. oral dose)		*Iron-Dextran Absorption (354 μg. oral dose)*	
Sex	*% of oral dose absorbed*	*Total μg. absorbed*	*% of oral dose absorbed*	*Total μg. absorbed*
F	29	14	49	173
F	34	17	48	171
F	36	18	50	177
F	44	22	37	131
F	65	33	48	172
F	82	41	54	192
F	88	44	78	278
F	—	—	45	160
M	40	20	46	162
Average	52	26	51	180

anemias with this compound. It could also be evaluated by measuring the incorporation of the Fe_{59} from the iron-dextran complex into the circulating red cells. Presumably, the transport and utilization of the iron from the iron-dextran complex would be similar to that of the iron from inorganic iron compounds. This assumption was not proved. If it is proved, oral iron-dextran should be an effective therapeutic agent in iron deficiency anemias.

Gastrointestinal side effects of the iron-dextran complex were not evaluated. The great majority of patients tolerate inorganic iron compounds without significant side effects. In these patients the iron-dextran compounds would not offer any advantage.

Summary. 1. The fractional gastrointestinal absorption of oral iron in the form of ferrous sulfate was measured in 8 healthy subjects, 7 women and one man. The range of absorption of a test dose of 50 μg. ranged from 29% to 88% of an oral dose of 50 μg., with an average of 52%. This represented an actual absorption of 14 μg. to 44 μg., with an average of 26 μg.

2. In the same 8 subjects, plus an additional woman, the absorption of iron from an iron-dextran complex varied from 37% to 78% of an oral dose of 354 μg., seven times the dose of ferrous sulfate. The average absorption was 51%. The actual absorption ranged from 131 μg. to 278 μg., with an average of 180 μg.

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SUMMARIO IN INTERLINGUA

Le Absorption Gastrointestinal del Complexo Ferro-Dextrano e de Sulfato Ferrose post Administration Oral

1. Le absorption fractional, in le vias gastrointestinal, de ferro oral, administrate in le forma de sulfato ferrose, esseva mesurate in 8 subjectos normal, i.e. 7 feminas e 1 masculo. Le procentages de absorption post un dose experimental de 50 μg variava inter 29 e 88. Le procentage medie esseva 52. Isto representava un absorption absolute de 14 a 44 μg, con un valor medie de 26 μg.

2. In le mesme serie de subjectos, augmentate per 1 femina, le absorption de ferro ab un complexo ferro-dextrano variava inter 37 e 78% de un dose oral de 354 μg (i.e., de 7 vices le dose de sulfato ferrose). Le absorption, medie esseva 51%. Le absorption absolute variava inter 131 e 278 μg, con un valor medie de 180 μg.

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THE EFFECT OF FERRIC CHLORIDE ON THE UTILIZATION OF CALCIUM AND PHOSPHORUS IN THE ANIMAL BODY

PEGGY REHM[1] AND JET C. WINTERS
Department of Home Economics, University of Texas, Austin

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There have been only a limited number of investigations on the problem of the effect of iron salts on the utilization of calcium and phosphorus. Waltner ('27) showed that adding 2% of reduced iron to McCollum's stock diet produced rickets in rats in 4 weeks. The blood serum of the rachitic rats showed low phosphorus but normal calcium, a condition similar to that found in human rickets. Cox, Dodds, Wigman and Murphy ('31), using guinea pigs and rabbits as experimental animals, showed that aluminum or ferric salts added to rations in such amounts that the aluminum or ferric ion was in excess of the phosphorus ion brought about drastic lowering of the bone ash and blood phosphorus. These investigators suggest that the reduction was due to the precipitation of alimentary phosphorus as unabsorbable ferric or aluminum phosphate. Using rats and adding ferric chloride to a non-rachitogenic diet, Brock and Diamond ('34) produced rickets of about the same degree of severity as is produced on the Steenbock rachitogenic diet. By substituting ammonium chloride for ferric chloride and finding that no rickets occurred, they showed that the ferric rather than the chloride ion was the causal factor. Deobald and Elvehjem ('35), working with chicks, demonstrated that the addition of large amounts of ferric citrate or aluminum sulfate to a diet which had been shown to be adequate for normal growth and bone formation brought about severe rickets in 1 or 2 weeks. Bone ash was reduced

[1] Died January 28, 1940.

almost 50% and the phosphorus in the blood serum was markedly lowered. These investigators suggest a possible danger from the use of large doses of iron in the treatment of hypochromic anemia. Day and Stein ('38) reasoned that if the addition of iron affected phosphorus metabolism, the addition of phosphorus ought to affect iron metabolism. They studied the effect upon hematopoiesis of different levels of calcium, phosphorus, iron and vitamin D in the diet. On diets low in calcium or iron, or high in phosphorus, rats developed anemia and polycythemia. Adding large amounts of iron chloride or calcium carbonate prevented the development of the anemia, but ferric phosphate and calcium phosphate were ineffective. The explanation offered for these results is that phosphorus combines with iron and interferes with its assimilation, but that, if sufficient calcium is present to combine with the phosphorus, the iron is free for assimilation. It is in this sense, Day and Stein think, that calcium is "a sparer of iron."

Two experiments have been reported that are not in line with the conclusions of Day and Stein. Kletzien ('38) reported that various calcium salts interfered with, rather than aided, storage of iron in young rats and that rats receiving phosphoric acid utilized more iron than controls receiving an equivalent amount of phosphorus as tricalcium phosphate. Shelling and Josephs ('34) also reported that calcium hindered iron utilization in rats. Further investigation concerning the metabolism of calcium, phosphorus and iron seems timely. The present experiments offer evidence of the detrimental effect of iron salts when added to an adequate synthetic diet under conditions of controlled food intake.

EXPERIMENTAL

In the first experiment two groups of rats, properly matched as to age, sex and weight, were used. One group was fed a standard, artificial diet, and the other the same diet supplemented with enough ferric chloride to combine with one-half the amount of phosphorus present in the ration. A group was

RESULTS

Table 1 gives the data obtained. This table shows that in spite of careful control of food intake, rats on the unsupplemented diet made greater weight gains than did those on the supplemented ration. This greater growth, in the case of the females, was made in spite of a lower food intake.

Total ash. As will be seen from table 1 the bodies of female rats kept on the synthetic diet supplemented with ferric chloride contained on the average about 0.9 gm. less total ash than did the bodies of female rats on the unsupplemented diet; the difference in the case of male rats amounted to 1.2 gm. This involves reductions of 20.0% and 25.5%, respectively. On the other hand, the percentage of total ash, based on net body weight, varied surprisingly little in the two groups of animals. This lack of variation is partly explained by the greater weight of the animals on the unsupplemented diet, but it should be remembered that this greater weight was attained without increase in mineral intake and would therefore indicate greater utilization of minerals. Variation in the amount of total ash in the individual animals of the same sex and on the same diet was not large and was not correlated directly with body weight.

Calcium. Reference to table 1 shows that as a result of the addition of ferric chloride there was a decrease in the calcium content of 0.29 gm. in bodies of the females and 0.36 gm. in the bodies of males or 23.9 and 28.6%, respectively. The average calcium, based on net body weight, in the bodies of female rats on the unsupplemented diet was 0.96%, and on the supplemented diet was 0.84%; for males, it was 0.91% and 0.81%, respectively. Individual variations in the calcium content of the animals in each group were small.

The decreases obtained in this experiment may be compared with those obtained by Sherman and Booher ('31) in an experiment in which the calcium content of the diet was varied from 0.16 to 0.32%. Sixty-day old male rats on a diet containing 0.16% calcium had a body content of 0.7%, while those on a diet containing 0.32% calcium had a body content

started in which enough ferric chloride was added to combine with all the phosphorus, but these animals developed a severe anorexia and lost weight so rapidly that it was impossible to use them for experimental purposes. The rats used were of good nutritional stock. They were placed on the diet at 22 days of age and all animals used weighed between 40 and 45 gm. at this time. Each group consisted of six males and six females. The animals were placed in separate galvanized iron cages with raised bottoms to prevent coprophagy.

The diet used consisted of:

	Per cent
Casein, unpurified	20
Cornstarch	56
Butterfat	8
Salt mixture (Osborne and Mendel)	4
Yeast, dried	10
Cod liver oil	2

Food intake for the two groups was kept the same by using the animals on the supplemented diet as controls since they had poorer appetites than those on the unsupplemented ration. Rats were weighed at 4-day intervals and adjustments in the quantity of food given were made at the weighing periods. Calculations at the end of the period showed that difference in food intake amounted to less than 0.5 gm. per day per rat. At the end of 3 weeks four animals, two from each group, were killed and analyzed for total ash, calcium and phosphorus. Results showed that a definitely lower ash content for the experimental rats had already developed and it was decided to terminate the experiment at the end of 1 month. At this time the rest of the animals were killed with ether, the digestive tracts removed and each animal analyzed separately for total ash, calcium and phosphorus. The percentages of total ash, calcium and phosphorus were then calculated on net body weight. Ashing was done in weighed silica dishes; a modification of McCrudden's method was used in determining calcium and the method of The Association of Official Agricultural Chemists for phosphorus.

TABLE 4

The effect of supplementing the diet with ferric chloride for a 30-day period on net body weight of cats, and total body ash, calcium and phosphorus

	EAT NO.	UNSUPPLEMENTED DIET							SUPPLEMENTED DIET						
		Net body weight	Total body ash		Total body calcium		Total body phosphorus		Net body weight	Total body ash		Total body calcium		Total body phosphorus	
		gm.	*gm.*	*%*	*gm.*	*%*	*gm.*	*%*	*gm.*	*gm.*	*%*	*gm.*	*%*	*gm.*	*%*
Females	1	133	4.412	3.61	1.209	0.91	0.818	0.62	—						
	2	122	4.542	3.41	1.232	1.00	0.818	0.67	—						
	3	121	4.524	3.64	1.226	0.99	0.804	0.65	—						
	4	121	4.385	3.62	1.218	1.00	0.810	0.67	—						
	5	130	4.379	3.36	1.186	0.91	0.786	0.61	—	—	—				
	11				—	—	—	—	110	3.632	3.30	0.916	0.86	0.658	0.60
	12				—	—	—	—	99	3.236	3.26	0.870	0.88	0.584	0.60
	13				—	—	—	—	118	3.634	3.15	0.937	0.81	0.666	0.58
	14				—	—	—	—	109	3.485	3.19	0.911	0.84	0.636	0.58
	15								120	3.786	3.15	0.955	0.80	0.684	0.57
Average		126	4.448	3.53	1.214	0.96	0.807	0.64	111	3.555	3.21	0.924	0.84	0.646	0.59
		Decrease from unsupplemented diet in per cent:								20.0	0.32	23.9	0.12	20.0	0.05
Males	6	141	4.756	3.37	1.278	0.91	0.866	0.61							
	7	141	4.811	3.41	1.276	0.91	0.877	0.62							
	8	143	4.803	3.36	[1]	[1]	0.869	0.61							
	9	141	4.676	3.31	1.286	0.91	0.856	0.61	—	—					
	10	145	4.824	3.33	1.293	0.89	0.874	0.60	—						
	16								110	3.482	3.16	0.897	0.81	0.629	0.57
	17								117	3.673	3.08	0.967	0.83	0.677	0.58
	18								116	3.636	3.13	0.937	0.81	0.652	0.56
	19								107	3.239	3.02	0.845	0.79	0.585	0.55
	20								120	3.747	3.12	0.968	0.81	0.696	0.58
Average		142	4.774	3.36	1.283	0.91	0.868	0.61	114	3.555	3.10	0.923	0.81	0.648	0.57
		Decrease from unsupplemented diet in per cent:								25.5	0.26	28.0	0.10	25.3	0.04

[1] Material lost.

of 0.87%. The difference of 0.17% was considerably larger than the difference of 0.10% obtained in the present experiment by adding ferric chloride to the diet. However, with female rats our difference of 0.12% was somewhat closer to the 0.16% obtained by Sherman and Booher.

Phosphorus. Data given in table 1 show that there was a decrease in phosphorus content of 0.16 gm. and 0.22 gm. in the bodies of female and male rats, respectively, as a result of the addition of ferric chloride to the diet. The corresponding percentage decreases were 20.0 and 25.3. The percentages of phosphorus in the bodies of female rats on the unsupplemented and the supplemented diets were 0.64 and 0.59, respectively. Corresponding figures for males were 0.61 and 0.57. The decreases in the percentage of phosphorus were not as striking as the decreases in the percentage of calcium. Just why calcium metabolism should be more drastically affected than phosphorus metabolism is a matter of conjecture. As was the case with calcium, individual variations in phosphorus content within each group were small.

In regard to total ash, calcium, and phosphorus, it may be pointed out that differences in the net body weight of animals in the two groups tended to minimize the detrimental effect of ferric chloride on calcium and phosphorus metabolism when expressed in percentage of net body weight; that is, the percentage decreases were quite small. Because of the controlled food intake, it is thought that differences in total ash, calcium, and phosphorus represent a truer picture than percentage differences. On the same calcium and phosphorus intake, much smaller amounts of these minerals were deposited in bodies of animals whose diet was supplemented by ferric chloride than in the bodies of animals whose diet was not so supplemented. It is probable that the interference with calcium and phosphorus metabolism was a causative factor in the marked anorexia and consequent poor growth of the animals on the ferric chloride supplement.

Effect of reducing cod liver oil. Because of the influence of cod liver oil on calcium and phosphorus metabolism, we

thought that it would be interesting to repeat the experiment, using smaller amounts of cod liver oil in the diet. It was also decided to adopt the pair-mate method in order to control the food intake even more accurately. In this method two animals of the same age, sex and weight are selected at the beginning of the experiment as pair-mates. The food intake for each day is carefully computed, and the animal on the smaller intake acts as control in determining the amount of food allowed his pair-mate. In this case it was, of course, the animals on the supplemented diet which acted as controls for those on the unsupplemented diet.

Four pair-mates, two on the supplemented and two on the unsupplemented diet, were studied over a period of 30 days;

TABLE 2

Net body weight of pair-mate rats

RAT	UNSUPPLEMENTED DIET NET BODY WEIGHT	RAT	SUPPLEMENTED DIET NET BODY WEIGHT
	gm.		gm.
2	135	8	104
3	130	9	94
4	126	10	98
5	130	11	101

the diet, in this case, contained only one-half the amount of cod liver oil as in the previous experiment. Net body weights are given in table 2. Again the rats on the unsupplemented diet made better weight gains than those on the supplemented.

Two animals on the unsupplemented diet were ashed together in the same silica dish, care being taken to select those of similar gains in weight and having similar food-intakes. The same procedure was followed with their pair-mates. Results are given in table 3.

In the previous experiment the average amounts of total ash, calcium, and phosphorus in the bodies of male rats on the unsupplemented diet were 4.774 gm., 1.283 gm., and

0.868 gm., respectively. When the cod liver oil was reduced by one-half, comparable amounts were 3.996 gm., 1.057 gm., and 0.745 gm. (average of nos. 2, 3, 4 and 5 in table 3). Thus, it will be seen that reducing the cod liver oil decreased the amounts of total ash, calcium and phosphorus in the body. This would be expected, since cod liver oil is known to improve retention of calcium and phosphorus. The percentage differences, however, between the animals fed the supplemented and unsupplemented diet were smaller when the cod liver oil was lowered. In the previous experiment

TABLE 3

Effect of ferric chloride on total ash, calcium, and phosphorus in bodies of pair-mate male rats

RATS		TOTAL ASH	CALCIUM	PHOSPHORUS
		gm.	*gm.*	*gm.*
Controls	2 and 3	8.010	2.119	1.498
Pair-mates	8 and 9	6.499	1.683	1.183
Decrease		1.511	0.437	0.315
Per cent decrease		18.9	20.6	21.0
Controls	4 and 5	7.972	2.109	1.483
Pair-mates	10 and 11	6.362	1.704	1.198
Decrease		1.610	0.405	0.285
Per cent decrease		20.2	19.2	19.2

the average percentage differences in total ash, calcium and phosphorus in the bodies of male rats were 25.5, 28.0, and 25.3, respectively. When the cod liver oil was reduced by one-half, comparable percentages were 19.5, 19.9, and 20.6 (average of decreases in table 3). This would indicate that the effect of adding ferric chloride is less drastic when small amounts of cod liver oil are supplied in the diet. From the standpoint of iron utilization these results indicate that iron might be better utilized when small rather than large amounts of cod liver oil are added to the diet. Less of the phosphorus is evidently bound as ferric phosphate; therefore more iron should be available.

SUMMARY AND CONCLUSION

A comparison of the amounts of total ash, calcium, and phosphorus in the bodies of animals on an unsupplemented diet with the amounts in bodies of animals on a diet supplemented with enough ferric chloride to combine with one-half the phosphorus of the diet was made, and it was shown that the addition of ferric chloride resulted in a considerable reduction in the amounts of total ash, calcium, and phosphorus at the end of 30 days. Similar experiments with like results have been reported in the literature, but in no other study has the food intake of the two groups of animals been equalized so that mineral intake of both groups was the same. In the present investigation the food intake of all animals has been kept approximately the same. In spite of this equalized food intake, animals on the unsupplemented diet gained more weight, and had larger amounts of calcium, phosphorus, and total ash deposited in their bodies at the end of the experimental period than animals with the ferric chloride supplement.

Although the analysis of each animal was carried out separately, discussion of results was based on group averages. Sex differences were not considered to be of such magnitude as to warrant separate discussion. Comparisons were made of percentage and total calcium, phosphorus, and ash in the bodies of both groups of animals. It has been pointed out that differences in net body weight of animals in the two groups tend to minimize the detrimental effect of ferric chloride on calcium and phosphorus metabolism, when expressed in percentage of body weight. Because of controlled food intake, it was thought that differences in total ash, calcium, and phosphorus represent a truer picture than percentage differences in arriving at a conclusion as to the effect of iron on calcium and phosphorus metabolism. Results of this experiment indicate that ferric chloride has a detrimental effect on calcium and phosphorus metabolism.

When the amount of cod liver oil in the diet was reduced by 50% the addition of ferric chloride resulted in a less drastic lowering of body calcium and phosphorus.

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Acute Intestinal Iron Intoxication

II. Metabolic, Respiratory and Circulatory Effects of Absorbed Iron Salts

By Kurt R. Reissmann and Thomas J. Coleman

IN THE PRECEDING PAPER[1] the true absorptive nature of acute intestinal iron poisoning was pointed out. Following oral or rectal administration of toxic doses of dissociable iron salts, it was found that excessive amounts of iron were absorbed through the anatomically intact intestinal mucosa, resulting in extraordinarily high serum iron levels within one hour after ingestion of the iron salts. These findings indicate that the frequently lethal outcome of acute iron poisoning is not the result of a local necrotizing effect of the ingested iron salts in the gut but is rather due to the toxicity of the absorbed iron.

Except for a marked capillary congestion, the autopsy findings in acute experiments did not provide any leads as to the site of action of the absorbed iron. In order to obtain such information, a number of vital functions were, therefore, studied in animals with intestinal iron poisoning.

Methods

Ferrous sulfate, ferrous gluconate and ferrous chloride were given by stomach or duodenal tube or by enema to dogs and rabbits. General procedures, dosage, and methods of serum iron determination were outlined in the preceding paper.[1] In addition, $FeSO_4$ was given to two dogs intravenously as a 4 per cent solution mixed with saline in doses listed in table 1.

The blood pH was measured in a Beckman pH meter immediately after the blood had been drawn under anaerobic precautions.

Sodium and potassium serum concentrations were measured in a Perkin-Elmer flame photometer, blood glucose according to Folin and Wu,[2] serum chloride according to Schales,[3] blood lactic acid according to Mendel,[4] blood pyruvic acid according to Bueding,[5] and serum citric acid according to Dickman.[6] Blood oxygen and plasma carbon dioxide content were determined by the manometric Van Slyke method, all blood samples being collected and handled under mineral oil.

Oxygen consumption, CO_2 output, respiratory rate and tidal volume were recorded by a modification of the Donald-Christie method[7] while the animal was breathing room air. Cardiac output was determined according to the Fick principle, the mixed venous blood sample being obtained through a cardiac catheter placed into the pulmonary artery. Blood pressure in the femoral artery was recorded by means of a Sanborn electromanometer and Poly-Viso direct writer. Plasma volume was measured with Evan's blue dye. Blood samples were drawn 15, 30, and 45 minutes after injection of the dye, and the spectrophotometrically determined densities were extrapolated to the time of injection. Relative cell volumes were determined in Wintrobe tubes, and a centrifuge correction factor of minus 5 per cent was applied to all hematocrit readings.

To detect possible abnormal hemoglobin derivatives, absorption spectra of the hemolyzed blood in 0.4 per cent ammonia were obtained in several dogs over the range from

From The Department of Medicine, University of Kansas School of Medicine, Kansas City, Kansas.

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Dog no.	Amount mg. Fe/ Kg. given	Route, iron salt		Before	Hours after iron administration ½	1	2	3	4	5	6	8	10	24
C19	150	Rectal $FeSO_4$	Fe μg. %	82			2720		3280		2520	2360	2160	
			pH	7.33			7.23		7.22		7.16	7.07	7.10	
			CO_2 meq.	22			19.0		17.5		11.5	10.9	8.6	
C11	200	Rectal $FeSO_4$	Fe μg. %	98	1100	1540	1830	1570		1360				
			pH	7.31	7.28	7.23	7.16	7.05		7.15				
			CO_2 meq.	28	25.7	25.2	21.4	15.6		14.2				
C22	235	Rect. Gluc.	Fe μg. %	70		700	1740	5780	8520	10280				
			pH	7.35		7.32	7.21		7.14	7.06				
			CO_2 meq.	24			14.1		11.1	7.6				
LC3	10	I.V. $FeSO_4$	Fe μg. %	116		7230	6170	4180	3310	2650	2200			372
			pH	7.36		7.14	7.10	7.16	7.21	2.21	7.22			7.33
			CO_2 meq.	24.5		20.3	19.5	16.8	16.2	18.2	20.5			26.5
C4	4 × 2.5 60′ interval	I.V. $FeSO_4$	Fe μg. %	111	1050	865	2780	5076	6280	4976	3560		1460	
			pH	7.36		7.26	7.22	7.22	7.14	7.04	6.93		6.85	
			CO_2 meq.	29.9		12.5	17.5	16.5	14.5	14.2	13.2		14.9	
318	150	Duod. $FeSO_4$	Fe μg. %	132		1564			961		904			248
			pH	7.34		7.30			7.16		7.26			7.29
			CO_2 meq.	25.1		22.1			16.0		14.7			21.8
C7	250	Duod. $FeSO_4$	Fe μg. %	116	1100	4560	7200	6600	6260		5820			
			pH	7.30	7.29	7.26	7.24	7.03	7.09		7.13			
			CO_2 meq.	22.2	19.5	17.8	17.8	13.5	11.0		11.2			
C5	250	Duod. $FeSO_4$	Fe μg. %	152	1150	5020	6400	4260		3460				
			pH	7.32	7.09	7.05	7.05	6.96		7.04				
			CO_2 meq.	21.4	19.0	18.5	16.2	16.0		14.8				

650 to 400 mμ in a Beckman spectrophotometer. Heilmeyer's[5] quotient 576/590 mμ was used as a quantitative index of methemoglobin.

Results and Discussion

(1) *Metabolic Effects*

In all animals a very marked hyperventilation developed within the first hour following ingestion of iron salts. This hyperventilation was found to be caused by a profound metabolic acidosis that developed rapidly and regardless of whether the iron was given by stomach tube, rectally, or intravenously* (tables 1, 2, 4).

The conversion of ferrous salts to ferric iron appears to be mainly responsible for the acidosis. Iron is, very likely, absorbed in the ferrous form, but when its plasma concentration exceeds the iron binding capacity of the plasma it is rapidly converted in the blood into the ferric form with formation of ferric hydroxide. Due to the insolubility of the latter and its tendency to form complexes, an increase in hydrogen ion concentration is to be expected. Such an effect was

* The acidotic effect of intravenous injection of iron salts was observed in 1880 by Meyer and Williams (Boston), working in Schmiedeberg's laboratory. They found a marked lowering of the blood CO_2 content following intravenous injection of iron tartrate. (Arch. Exper. Path. Pharmak. *3*: 70, 1880.).

demonstrated in vitro when $FeSO_4$ was added to blood kept in equilibrium with 5 per cent CO_2 in 95 per cent O_2. Blood pH and plasma CO_2 were determined before and sixth minutes after adding the iron, allowing this time interval for a conversion of Fe^{++} to Fe^{+++}. The following results were obtained:

	pH	CO_2	Fe
		meq./L.	*μg./100 cc.*
Before adding Fe	7.36	24.4	118
After adding 10 mg% Fe^{++}	7.25	23.0	10,367
After adding 20 mg% Fe^{++}	7.14	18.8	19,920

In these in vitro experiments the CO_2 tension was kept constant. In vivo the pH change which can be expected from an increment of 10 mg. per cent in serum iron will be smaller because of a compensatory hyperventilation with increased dissipation of CO_2 and removal of carbonic acid. Yet it will be noted in table 1 that in the iron poisoned animal much greater pH changes occurred and at serum iron levels which were considerable lower than those in the in vitro experiments. It has been pointed out already, that the serum iron level in these animals is not a criterion of the total amount of iron absorbed because iron is constantly transferred from the blood into the extravascular compartment. As the conversion of this iron from the ferrous into the ferric form takes place in the blood, the acid liberated in this process will progressively titrate the plasma buffers and this accumulative effect cannot be judged from the serum iron values.

An increase in organic acids was found as an additional factor in the greater in vivo acidotic effect of iron salts. Lactic and citric acid increased progressively in the blood of the iron poisoned animal as seen in table 2, which is a representative example of several experiments. Muscular activity or convulsions could be ruled out as the cause of the increase in lactic acid. The role of the capillary dilatation and congestion in the development of the acidosis is difficult to evaluate. From respiratory and circulatory data (vide infra), it appears that the accumulation of organic acids occurred earlier than the circulatory and respiratory failure and that circulatory anoxia was not a decisive factor in the development of the acidosis, at least not during the first hours following iron administration. It is, therefore, suspected that in iron poisoning the catabolism of the organic acids is interfered with, possibly by an effect of iron ions upon enzymes in the Krebs cycle. Such intracellular toxic effect of iron found support in the results

TABLE 2.—*Dog C17, 350 mg. Fe/Kg. Body Weight by Rectum as $FeSO_4$*

Time in hours	Serum iron, gamma %	Blood pH	Plasma CO_2, meq. L	Blood glucose, mg.%	Blood lactic acid, mg. %	Blood pyruvic acid, mg. %	Serum citric acid, mg. %	Serum Na, meq. L	Serum K, meq. L	Serum Cl, meq./L
Before	150	7.29	21.9	111	16.0	2.23	3.33	144	4.5	104
1	10,120	7.09	17.5	153	23.0	2.02	7.78	141	4.9	110
2				136	32.0	1.81				
3	10,700	7.06	12.0	170	28.5	2.46	9.16	142	5.1	110
4				258	36.0	2.10				
5	10,320	7.02	8.5	201	39.5	2.76	16.89	145	5.3	114

Several experiments were made in vivo administration of sodium bicarbonate failed to prevent the fatal outcome of iron poisoning.

As the acidosis occurred invariably both in dogs and in rabbits, it seems reasonable to assume that it was likewise present in children with iron intoxication, although it apparently escaped observation in the reported cases. While a rapid respiration is commonly mentioned in the case histories, we could only find one CO_2 determination (15 meq.)[9] in the reports.

When the animal survived the acute phase of iron intoxication, the pH returned to normal values within 24 hours. As these animals did not excrete significant amounts of iron during this time, these findings suggest that the iron ions must be neutralized or bound somewhere in the tissues and that normal metabolic cell functions are restored.

(2) Circulatory and Respiratory Effects

The hemoconcentration suggested by the increase in hematocrit values was confirmed by the determination of the plasma volume by means of the dye method. As seen in table 3, the plasma volume decreased from 577 cc. before, to 439 cc. two hours after, and to 401 cc. four hours after iron had been administered. A similar trend was seen in two other dogs. As the urinary output was less than 50 cc. and the amount of fluid usually seen in the intestinal lumen after iron poisoning was not excessive, the most likely explanation of the decrease in plasma volume is a shift of fluid from the vascular compartment into the interstitial and possibly into the intracellular compartment. The increase in total cell volume probably is not real because the cell volume was not measured directly but was derived from the plasma volume and the venous hematocrit, and it is likely that in the state of marked capillary congestion, the relation between venous hematocrit and body hematocrit was markedly altered.

In spite of the reduced plasma volume, a normal blood pressure was maintained for several hours until the final collapse occurred, as seen in table 4. The cardiac output dropped progressively and the heart rate increased markedly, resulting in very marked changes in the stroke volume which dropped from 10 cc. to 3.3 cc. This decrease in cardiac output is probably not so much a direct effect of the iron ions upon the heart muscle but rather is secondary to a diminished venous return. The latter is due to congestion in the capillaries and venules and partly due to the reduction in plasma volume. A marked capillary congestion and increased capillary permeability throughout the body was found at autopsy in all animals. It could not be decided whether this capillary dilation

TABLE 3.—*Plasma Volume in Dog CO6 before and after Administration of 200 mg. Fe per Kg. Body Weight by Duodenal Tube. Weight of Dog 11.80 Kg.*

Time after Fe administration	Serum Fe, gamma %	Blood pH	Plasma CO_2 meq./L	Hematocrit corrected	Plasma volume, cc.	Cell volume, cc.	Blood volume, cc.
1 day before	112	7.27	21.2	46.0	577	491	1068
2 hours after	2350	7.17	21.0	55.5	439	559	998
4 hours after	3200	7.15	16.2	58.3	401	560	961
24 hours after	462	7.34	18.7	54.5	467	559	1026

TABLE 4.—*Circulatory and Respiratory Function in Acute Iron Poisoning, 11.6 Kg. Dog, Nembutal Anesthesia, 200 mg. Fe/Kg. Body Weight by Enema*

Time after Fe admin.	Serum Fe	pH	Hemoglobin Gm.	Heart rate, min.	Arter. pressure mm. Hg, syst./diast.	Arter. pressure Mean	Cardiac output, L/min.	Stroke volume, cc.	Breathing volume, L/min.	O_2 consumption, cc/min.	CO_2 output, cc/min.	Resp. rate, min.	O_2 saturation, % Art.	O_2 saturation, % Vein*	CO_2 content, vol. % Art.	CO_2 content, vol. % Vein*
Before	165	7.29	12.6	120	170/105	115	1.21	10	1.89	94	56	9	95	63	44.8	40.6
1h	9,757	7.13		195	150/110	120			3.30	68	78	12				
2h	10,437	7.09	14.9	200	150/110	120	1.18	5.9	5.00	73	80	16	93	57	24.5	31.1
3h	10,297	7.07		203	145/115	125			5.70	72	76	19				
4h	10,097	7.09	16.4	230	135/110	125	0.76	3.3	5.20	67	71	20	92	53	19.6	28.9
5h	10,141	7.03		218	130/105	110			5.04	62	62	24				
6h	10,022	6.72		95	75/42	58			Animal dying							

* Mixed venous blood obtained by catheter from pulmonary artery.

was the result of the acidosis and its underlying disturbance in cell metabolism, or whether the high serum iron concentration directly affected the capillary system.

The greatly increased breathing volume (from 1.89 L to 5.7 L per minute) reflects the acidotic stimulation of the respiratory center. The CO_2 output rose markedly as a result of the accumulation of the organic acid and the RQ of greater than 1 reflects the increased CO_2 elimination rather than the CO_2 production. The O_2 consumption rose slightly above the baseline level in spite of the described interference with the oxidative breakdown of the organic acids. This slight rise is probably due to the greater energy expenditure in the increased ventilatory efforts and indicates that at least during the first four hours no significant degree of anoxia was present. The arterial O_2 saturation decreased from 95 to 92.5 which can be entirely accounted for by the effect of the lowered pH upon the O_2 dissociation curve of the hemoglobin (shift to the right).

A possible formation of methemoglobin in the blood of children with iron poisoning has been discussed[10] but not demonstrated. The blood in eight experiments and at various stages of iron intoxication was examined spectrophotometrically. No deviations from a normal oxyhemoglobin spectrum were found, and in all instances Heilmeyer's quotient 576/590 was found to be above 3.6, indicating that no significant amounts of methemoglobin were present.

In the experiment presented in table 1, the dog suddenly stopped breathing at six hours after Fe administration. Blood pressure and heart rate dropped rapidly, and the dog died in respiratory failure, which was the direct cause of death in most experiments.

SUMMARY

Following oral or rectal administration of toxic doses of dissociable iron salts in dogs and rabbits, the rapidly and excessively absorbed iron produced a profound metabolic acidosis with blood pH values as low as 6.7. The acidosis was mainly due to the hydrolysing effect of ferric ions and partly due to an increase in lactic acid and citric acid. The latter findings suggest a possible interference of the [illegible] with enzymes in the Krebs cycle.

The respiratory changes were those seen in metabolic acidosis: greatly in-

creased respiratory rate and minute volume, lowering of the blood CO_2, excessive CO_2 output. The cardiac output decreased progressively due to diminished venous return, but a normal blood pressure was maintained by arteriolar constriction until the final collapse occurred, which was preceded by respiratory failure.

A marked capillary congestion and increased capillary permeability were noted, the latter possibly being the result of a direct action of the high non-protein bound serum iron upon the capillary wall. The increased capillary permeability caused a reduction in plasma volume and hemoconcentration.

No abnormal hemoglobin derivatives were found.

Summario in Interlingua

Post administration oral or rectal de toxic doses de dissociabile sales de ferro in canes e conilios, le rapide e excessive absorption de ferro resultava in un forte acidosis con valores del pH sanguinee abassate usque a 6,7. Le acidosis esseva debite in parte al effecto hydrolysante de iones ferric e in parte al augmento de acido lactic e citric. Iste ultime constatationes suggere le possibilitate de un interferentia del ferro absorbite con enzymas del cyclo de Krebs.

Le cambiamentos respiratori esseva illos observate in acidosis metabolic: forte augmento del rapiditate e del volumine-minuta de respiration, reduction del CO_2 sanguinee, excesso de discarga de CO_2. Le rendimento cardiac decresceva progressivemente in consequentia del reducite retorno venose, sed un pression sanguinee normal esseva mantenite per constriction arteriolari usque al occurrentia del collapso final. Isto esseva precedite per syncope respiratori.

Un alte grado de congestion capillar e un augmento del permeabilitate capillar esseva observate. Iste ultime esseva possibilemente le resultato de un action directe super le parietes capillar per le alte contento de ferro seral non ligate a proteina. Le augmentate permeabilitate capillar causava un reduction del volumine plasmatic e del hemoconcentration.

Esseva observate nulle abnormalitates in derivatos hemoglobinic.

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A New Concept of Cancer: Cause and Cure*

JOHN C. ROMMEL, M.D.
PHILADELPHIA, MISS.

THE BIG PROBLEM in cancer is to locate the beginning of the disease, early in the history of the patient, before the mushroom growths we know as cancer have developed. This condition that we call cancer is the terminal stage of the disease. Where shall we find the beginning? As a result of study and treatment I am of the opinion that cancer is an iron-deficiency disease and can be cured by the administration of iron, preferably ferrous sulphate. It is not as easy as it sounds in that statement. The longer the disease has existed, the more difficult it is to cure. The tumor stage is quite resistant to the administration of iron. In fact, Dr. Russell Haden (in Surgical Clinics of North America, October, 1939) says the presence of cancer prevents the body from making use of iron administered. My patient, S. T., case 2, reported herein, bears out that statement. Haden also says that after the removal of the cancer the corpuscles can then take up the iron.

How does cancer begin? Cancer begins with vague disturbances for which no cause can be positively determined, and runs on, year after year, while the patient goes from doctor to clinic and from clinic to doctor, trying to find someone to grasp the situation and cure the difficulty. Many patients who are found in this category are the beginning cancer cases.

In some patients (those who develop abdominal cancer) vague abdominal discomfort is an early complaint. The gastro-intestinal tract is examined and studied. Indigestion, loss of appetite, decomposition of food, sick feeling in the stomach, constipation, abdominal distention, fecal impaction, bearing-down distress, are included among the complaints.

Frequently there is actual pain in the abdomen. Later, more or less continuous pain is present just below the ribs, extending across the abdomen, usually in the areas of stomach, liver and gallbladder.

These cases are now being treated for the symptoms of which they complain, such as indigestion, constipation, gallbladder disease etc.

Skin cancer shows as a scaling keratosis as a brown spot. There is a thickened, reddened spot or pink nodule in some cases. This grows slowly and finally ulcerates. The ulcer is shallow and the base indurated. This lesion is painless. Metastases occur. It also spreads in the dermis.

Leucoplakia of the lips and mouth are frequently the beginnings of the outbreak in those areas. On the tongue the areas become thickened, warty and eroded. Enlargement of the cervical lymph glands occurs.

The Plummer-Vinson syndrome concerns cancer of the neck and will be discussed later.

Cancer of the lung is mentioned by Scupham. This also will be discussed later.

Cancer of the breast follows prolonged low hemoglobin and the development of the lumps in the breast which progressively enlarge.

Leucoplakic areas may be found in the vagina preceding the development of vaginal cancer.

In the bladder hematuria may occur. Later leather-like consistency is detectible, found by palpation over the pubis. This condition is preceded by bladder discomfort, with symptoms of cystitis. The usual treatment for cystitis does not help the patient with this condition. The doctor is puzzled then as to how to treat the bladder until he palpates the leatheroid bladder wall or else finds growths.

In the testicle painless enlargement may be found.

On the penis there may be an open sore, mole or bleeding wart.

I began the study of cancer two years ago, when I had a patient, whose history I shall give later. I also had a relative in another city afflicted with it at the same time. I therefore had the subject continually before me, and it gave rise to a multitude of thoughts on the why and wherefore.

In the spring of 1938, this relative in a distant town was treated by x-ray for cancer. I called to see him frequently. In the fall, about

*Read before the North Branch of the Philadelphia County Medical Society, May 16, 1940.

October of that year, while riding home after visiting him, I wondered why we have so much cancer today; so much more than formerly. I wondered if, in the past, we had been giving some remedy that cured the patients and prevented cancer, and were not using it so much today. So I thought of iron, which was used much, 25 years ago, but not as much now, and of ferrous sulphate as the preferred form. Why ferrous sulphate? Most persons need some sulphur in their system, and the consensus of opinion seems to be that ferrous sulphate is the best iron salt to use. So I began using it on patients, with the results shown in the following case reports:

CASE 1

A. M. Age 66, average build.

On May 23, 1938, sent for me and said she has had vaginal bleeding since August, 1937, a period of ten months. The bleeding has been quite profuse lately. She seemed almost exsanguinated. I prescribed ergotrate t.i.d. and sulfanilamide, 10 grains t.i.d. The ergotrate stopped the bleeding after several weeks' use. I was of the opinion that cancer was bacterial in origin, but the sulfanilamide had no beneficial effect and I discontinued it.

On June 16 examination disclosed an enlarged uterus. She was referred to the Jeanes Hospital. On August 12, 1938, Dr. Teahan wrote me as follows:

"Microscopically the tumor is a highly malignant adenocarcinoma." She was treated at the Jeanes Hospital and returned home for a while. She reported to the Jeanes Hospital at intervals for examination and treatment.

On August 26 she complained to me of pain in the rectum. The anal area was sore to pressure. Interior of rectum did not pain. On September 9 glycerite of tannin was applied to anus, for ulceration. On September 16 she was dizzy, weak, and passing blood by rectum. Kaopectinate was prescribed.

I then tried another remedy to control the cancer. I prescribed hormotone without postpituitary and quinine and urea hydrochloride. So at least some hormones are not particularly valuable in this disease. Perhaps I should have used the hormotone for a longer time.

On November 11 iodized calcium, ½ grain, and ferrous sulphate 5 grains, was given in capsule three times a day. She took this until she returned to Jeanes Hospital, where on December 3, 1938, hysterectomy was performed. I saw the uterus after the excision. The cervix was the only part that showed any disease. The body of the uterus showed no sign of cancer.

Dr. Wammock, of the Jeanes Hospital, wrote me as follows, on December 30, 1938:

"Mrs.———— was discharged from the hospital on 12 23 38 after an uneventful recovery from a panhysterectomy which was performed on 12 3 38. The histological examination of the uterine specimen did not show any evidence of carcinoma and this should give a very favorable prognosis."

I would not claim that the remarkable change was due to the ferrous sulphate. The radium and x-ray treatment had positively good effects. But I felt encouraged to continue the treatment of any further cases that might come to me.

The patient returned to the hospital 10 months later with inoperable carcinoma of a rib below left breast, showing that metastases had spread beyond the scene of the area treated by the x-ray, radium and hysterectomy.

Another case, not treated surgically, carries out the idea much better.

CASE 2

S. T., aged 76, short and thin, came for treatment on April 11, 1939. She complained of having had bearing-down distress in abdomen for many years. Now has sick feeling in stomach. Was treated for piles two years ago. She is a poor sleeper. Wakes up early; then gets sleepy in daytime. Constipated. Takes pills. Eats nearly everything. Blood pressure 160/84. Pulse, 82.

She presented the characteristic appearance of abdominal cancer. Her abdomen was highly puffed, the skin was stretched taut, bluish in color, glazed and edematous, and tender to touch. She weighed 87½ pounds.

Cases like this I used to send to hospital for operation, and some of them died in a few weeks. I diagnosed the condition as cancer in stomach, gallbladder, and liver, with metastases throughout the mesenteric glands. I had seen similar cases that later were autopsied, and some that demonstrated cancer at operation.

She was given HCl, eserine, and elixir peptenzyme for digestive system, Pill A.B.S. & C. for constipation, and bromides for sleep.

Along with general treatment, ferrous sulphate, 5 grains, 3 times a day was given. She is still taking the ferrous sulphate. Vitamins were also given her at times.

The abdomen soon began to improve. The distension decreased, the pink color gradually returned, and glazed skin gave place to normal appearance, and the edema remained long after the other symptoms were cleared up, but finally disappeared. She still has a small amount of distension in lower abdomen; but it is gas in the intestines.

On the first test her hemoglobin was 80. Seven months later it was still 80, showing the difficulty of improving the Hb content as stated by Russell Haden.

The lady who referred her to me said that formerly her clothes were placed around her room in more or less disorder, before treatment; but after a few months' treatment she kept her room in good order.

Her only complaint now is some neuritis, which is being treated. So that in spite of the failure to improve her Hb content, she made progressive improvement in her symptoms and condition.

CASE 3

J. D., short, but not thin. At age 25 (in 1930) gave her weight as 100 pounds. Had no gain in weight in the preceding 10 years.

Complained of weakness, fickle appetite, indigestion. Main complaint: pain in abdomen.

In May, 1937, at Jefferson Hospital where she went on account of the abdominal pain, had an examination, which showed: hemoglobin 74, erythrocytes 3,700,000, leucocytes 7400. Wassermann and Kahn negative. The diagnosis of her trouble concluded that there was chronic gallbladder disease.

On December 17, 1938, she complained to me of pain in left chest and abdomen, swelling of abdomen, easily tired, and got out of breath. She was given sulfanilamide, 5 grains, 3 times a day, and ferrous sulphate and calcium iodized, 3 times a day. She had albumin in her urine and was put on a vegetable diet. She later admitted that she did not adhere any too well to her diet.

On November 16, 1939, she returned with an enormously swollen pancreas, which was exceedingly painful, especially on efforts to palpate and delimit it. She said she was stiff in hands, shoulders, body, etc., and also ached.

She said her abdominal illness began with abdominal flue in 1918, and she had had trouble in abdomen ever since. Hb 60. In 2½ years the Hb had fallen from 74 to 60. Ferrous sulphate was administered 3 times a day without the iodized calcium.

On November 28 the abdomen was less swollen. Several masses were felt below pancreas. Pancreas less tender. Said lower masses were worse (more painful). Urine was 1032, amphoteric in reaction, no albumin, no sugar. I anticipated diabetes from the pancreatic inflammation, but it did not occur. The pancreas slowly resolved, and the specific gravity dropped down to normal figures.

She is much improved and continuing the treatment with 5 grains of ferrous sulphate three times a day. Her treatment leads to the suggestion that ferrous sulphate might cure a diseased pancreas in diabetes.

CASE 4

H. B., aged 85, tall and thin. Left breast enlarged to size of large orange, ulcerated and bleeding. Hb 20. Said the breast trouble had been coming on for 17 years. She was given ferrous sulphate, 5 grains three times a day. In the course of a month the ulcers were healing on the surface of the breast. She is discouraged, but is still taking the medicine.

I no longer use the iodized calcium in these cases. No doubt, many patients need iodine but I tried out the iron alone, and believe it is the main agent in this disease. No doubt these patients need all the elements and vitamins, and I believe they would be helpful and not harmful.

CASE 5

C. H., 65. On December 13, 1939, stomach had been bad for week, had pain in ear, hard of hearing. There was inflammation of the right aural canal, with a bloody mass present and a similar condition in right nostril. Prostigmin was injected for the deafness, and ferrous sulphate given for the bleeding masses. His hemoglobin showed 60.

On December 4, the ear was improved, and no further bleeding from the nose since the previous visit. On December 12, there was still some blood in right aural canal. None in nose. On December 14, there was some blood in the nose. On December 27, there was blood in left nostril. No blood found thereafter.

I think this is a precancerous case. He is still taking ferrous sulphate. His bloody masses in nose and ear made me suspect beginning cancer.

The Plummer-Vinson syndrome, described in J. A. M. A., November 11, 1939, p. 1814, concerns patients with "hypochromic anemia with or without achlorhydria, dysphagia, and chronic inflammatory changes in the mouth.

pharynx, and upper end of the esophagus. According to Ahlbom at Radiumhemmet in Stockholm, the syndrome brings with it a special liability to cancer in those structures. As a rule, the afflicted women are poorly developed and poorly nourished; weakness and anemia may have existed for years. . . . Iron in large doses leads to improvement; even to apparently complete recovery; only too frequently the anemia returns and the dysphagia may become more or less continuous for years."

The answer to this is to continue the iron treatment, even after apparent recovery.

Concerning cancer of the lung, G. W. Scupham, said: "Whenever pulmonary symptoms, particularly dyspnea and pain, are out of proportion in their severity to x-ray or physical findings, bronchiogenic carcinoma is likely." (J. A. M. A., December 10, 1939, p. 2398). In this quotation is shown the uncertain condition in which cancer is found. You rule out everything else, and then you diagnose cancer. The hemoglobin test will be a striking point in conjunction with these symptoms. And the iron medication improves the patient and, at least in some cases, not the hemoglobin content.

The Bulletin of Practical Ophthalmology for January, 1940, p. 23, records an epithelioma of the tongue. The writer says: "This man, who was aware of the presence of leucoplakia 20 years ago, faithfully went to have it treated with the cautery and caustics." The writer indicates that he considers the leucoplakia a precancerous condition. I believe he is right.

SUMMARY

Cancer is a long-lived disease. It begins insidiously. Fugacious pains, not accountable in our ideas of specific pathology, are more or less in evidence.

In my view the beginning is a deficiency of iron, as shown by the hemoglobin test. The Tallquist color test is a good guide.

All the patients I have seen have a marked deficiency of hemoglobin, ranging from 70% to 30% and one showed 20%.

In the cases of cancer where you find tumor growth, you will probably encounter metastases. I believe they are present in all such cases, for the anemia is necessarily present throughout the body. Removing the visible part does not cure the disease, for metastases later take off the patient. Metastases, as you now, may occur in any tissues and in any part of the body.

The diagnosis rests on finding pain, aches, disturbances, ill feeling, dysfunction, indigestion, constipation, lowered Hb, in patients whose symptoms are not otherwise explainable. Should this state continue long enough, the condition we call cancer sets in. How many years that requires cannot, at this time, be stated.

One of the first things to do, then, is to make a hemoglobin test. The earliest forerunner of cancer is a lowered hemoglobin in the body. Then supply iron in sufficient quantity and proper condition to bring up the hemoglobin to normal, and you prevent cancer. No doubt, a small amount of copper should be administered, which makes the iron in the blood more easily taken up by the hemoglobin.

In any event, even in late stages, administer ferrous sulphate. If the disease has not gone too far, you should cure the patient. Tonics and vitamins are needed to bolster up the ailing body, and should be vigorously given.

Cancer favors the women because they lose so much blood and their hemoglobin drops. They should all be taking ferrous sulphate.

If all persons with deficient hemoglobin are promptly given ferrous sulphate—and other remedies as needed—there should be no more cancer.

Primarily the iron deficiency is due to a lack of iron in the diet. When the hemoglobin is reduced, however, pharmaceutical iron must be administered to make up the deficiency. Attempts to cure cancer with diet alone would fail.

The best hope in these cases is in discovering the precancerous condition. Making the hemoglobin test is the first thing to do. With a deficiency of hemoglobin the patient is at least in the danger zone and appropriate treatment with ferrous sulphate should be given. Long continued deficiency of hemoglobin calls for prompt and vigorous action. The beginning of the various symptoms of illness described in connection with the cases reported and of other symptoms for which no definite cause can be assigned demands iron, vitamins, digestive remedies and other supportive measures. The persistent low hemoglobin record, improved little or none by administration of iron, is found in late stages as shown by Russell Haden and the case of S. T., described herein.

Then cancer can be prevented and in some cases cured. When the terminal stage attacks and destroys some vital organ or organs, the outlook is most serious. Only some points in the life history of cancer are described here, and much work remains to be done in this line of investigation.

The thought arose that 10 mg of iron oxide per kilogram of body weight in the dog were already outside of the limits which must be maintained in order to see regular reduction in the oxidation which was observed by Wada in rabbits at a dosage of 2.5 mg iron oxide. Then, maintaining exactly the test conditions as used by Remesow, I then investigated on two healthy dogs which had never before been subjected to any tests the effect of the peroral administration in my dog 1 of 2.5 mg and in my dog 2 of 5 mg of active iron oxide on the metabolism.

I point once again to the fact that all of the tests carried out above were done with the active iron oxide prepared according to the method of Baudisch and indeed the tests by Wada and Remesow with iron oxide which was prepared ad hoc in the local laboratory according to Baudisch, and my own experiments with active iron oxide which was produced by Dr. Baudisch himself in the Rockefeller Institute in New York and was kindly made available to us.

I anticipate here the result of my own tests and then in the experiment part of my work I leave the test protocol.

In dog 1 the peroral administration of 2.5 mg of active iron oxide per kilogram of body weight ledd to the following metabolism changes:

1. The utilization of food improved in the intestines since the daily excrement N and excrement C values dropped.

2. The N secretion through the urine dropped.

3/ The protein oxidation dropped by about 25%.

4. The C secretion through the urine rose.

5. The urine quotient C:N increased as a result of the increasing C excretion and the decreasing N secretion through the urine.

6. The body weight remained constant.

In dog no. 2 the peroral administration of 5.0 mg of active iron oxide per kilogram of body weight led to the following phenomena:

1. The food utilization in the intestines improved since the daily fecal N and fecal C values fell.

2. The N secretion through the urine dropped.

3. The protein oxidation dropped by about 14%.

4. The C secretion through the urine increased.

5. The urine quotient C:N rose as the result of the increasing C secretion and the decreasing N secretion through the urine.

6. The body weight remained constant.

7. A long after-effect is detectable after suspension of the iron medication.

We can see from all of this that the results of the tests on both dogs were basically identical. In the case of the appropriate dosage, which we consider to be 2.5 to 5.0 mg of active Baudisch iron oxide per kilogram of body weight and per diem in peroral application, there occurs with an improvement in the food utilization in the intestine, hence with increased C and N resorption, a great drop in protein oxidation and to the extent of the increased value of the dysoxidizable C urine also an at least qualitative reduction in the oxidation of the N-free C-containing substance (fats and carbohydrates) ., whereby this phenomenon should not be caused by an increase in the fat and carbohydrate oxidation in the quantitative point of view.

When we compare my tests with those of Wada and Remesow and when we refer to the gas exchange tests of Arnoldi on rats when feeding with this active iron oxide (Arnoldi found as an after-effect an increase in the O_2 utilization with constant respiratory quotients) , then we reach the conclusion that the regular and primary metabolism effect in the optimal dosage of active iron is the reduction in protein oxidation and perhaps also a reduction in the overall conversion. To what extent this results in a lung gas exchange requires additional study. In the case of dosage which is not optimal, i.e. an excessive dosage with respect to the optimum, and perhaps as a function of the general nutritional conditions and the individual adaptation of the organism to the active iron , we see during or at the end of the iron administration increases in the oxidation of the N-free substance and lastly also exceptionally increases in the protein oxidation.

Experiment Part

In the test use was made of two healthy dogs which had not yet been used in experiments: dog no. 1 and no. 2. The diet was composed daily of the same amount of wheat protein, rice, yeast, butter and cooking salt so that at a calory intake of 80 calories per kilogram of body weight with a total N intake of 7.4588 g per day per dog in the case of dog no. 1 and 5.83 g in the case of dog no. 2 this guaranteed constant weight and a slightly positive N balance. The daily N balance of dog no. 1 was + 1.2505 and for dog no. 2 +0.18.

In addition each dog was given daily cell salt (see Table III). The entire test in the case of dog no. 1 lasted 15 days and in the case of dog no. 2 the entire test took 45 days. The carbon was determined according to the method of Tangl-Keresky in the modification of Gomez. Double analyses were regularly made and only conforming results were used. The nitrogen was determined according to Kjeldahl. The daily urine quotient C:N was calculated from the urine amount spontaneously secreted in 24 hours. Each of the two dogs were kept in a preliminary period which amounted to 8 days each for dog no. 1 and for dog no. 2 and during that period there was practically an absolute constancy of the C:N quotient. Only then was the main period started. In the main period iron was given. In the case of dog no. 2 after the main period there was applied an after-period with iron medication in order to study the after-effect. Dog no. 1 was given 2.5 mg of active iron oxide per kilogram of body weight, dog no. 2 received 5.0 mg.

The individual periods were separated from one another by Carmin which was given in the morning with a small amount of food. Carmin-containing excrement was not used in the analysis. The excrement was prepared according to the method of Poda, i.e. dried up to constant weight, the dry excrement amount was determinedpounded and ground and then used for the analysis. The excrement determinations were only made periodically. The daily N balance was determined from the N-excrement amount calculated per diem for the period average, the amount of N added through the food and the N urine amount calculated per day for the period average. The percentage protein oxidation was calculated in the following manner: from the N food amount determined per period the period N-excrement amount was subtracted and the result was referred to the period N-urine amount. An example for dog no. 1 can explain this quickly: Let there be 59.6704 g food periods N, 6.4260 g periods excrement N, 43,2424 g urine periods-N; from this we obtain: 53.2444:43.2424=100:X.

$$X = \frac{43.2424 \cdot 100}{53.2444} = 81.2\% \text{ N utilization for the pre-period.}$$

The urine quotient (factor) for the period average was calculated from the total urine C amount divided by the total urine-N amount in the period.

Table 1

Food table Dog no. 1. Weight 11100 g on February 9 1927.

Food intake about 80 calories per kilogram body weight.

Daily: 1. wheat protein	= 45.0 g	= 6.0273 g N	= 169	calories
2. rice	= 95.0 g	= 1.1704 g N	= 269	"
3. yeast	= 12.0 g	= 0.2611 g N	= 42	"
4. butter	= 45.0 g		= 418	"
5. cooking salt	= 1.5 g			
6. cell salt	= 1.5 g			
Totalling	= 7.4583 g N	=	598	calories

Table 2

Food table Dog no. 2. Weight 6600 g on February 9 1927.
Food intake about 80 calories per kilogram of body weight.

Daily: 1. wheat protein	= 35.0 g	= 4.6879gN	= 131	calories
2. rice	= 80.0 g	= 0.9856 gN	= 227	"
3. yeast	= 6.0 g	= 0.1530 g N	= 21	"
4. butter	= 20.0 g	=	188	"
5. cooking salt	= 1.5 g			
6. cell salt	= 1.5 g			
Totalling	= 5.8265 N	=	567	calories

Table 3 Cell salt mixture.

Calc. phosphor.	= 100.0	Ferr. citr.	= 30.0
Mgn. citr.	= 100.0	pure iodine	= 0.05
Cal. chlor.	= 15.0	cal. iod.	= 0.1

Table 4. Dog no. 1. Pre-period

1= date; 2= body weight, 3= 24-hour urine amount 4= urinc C; 5= urine N; 6= C:N; 7= dry excrement amount per period; 8= daily N balance in the period average; 9= remarks; 10= Diet see table 1. During the period no protein, no sugar in the urine, the diet was taken in full daily. 11= Carmin. From March 3 in the afternoon 2.5 mg active iron oxide per kilogram of body weight.

1 Datum 1927	2 Körpergewicht g	3 Harnmenge ccm	4 Harn-C	5 Harn-N	6 C:N	7 Trockenkotmenge pro Periode g	8 Tägliche Stickstoffbilanz im Periodendurchschnitt	9 Bemerkungen
28. II.	9 [illegible]	740	3.1121	7.4549	0.416	101,0	+ 1.2505	Nahrung s. Tabelle 1. Während d. Periode kein Albumen, kein Zucker im Urin. Nahrung wird tägl. ganz genommen
24. II.	9 850	710	2.9419	6.1075	0.481		+ 1.2505	
26. II.	10 170	365	2.4718	5.4918	0.444		+ 1.2505	
27. II.	10 050	880	2.9776	5.7211	0.480		+ 1.2505	
28. II.	10 020	780	2.8743	5.4218	0.484		+ 1.2505	
1. III.	10 100	490	1.2481	3.2278	0.386		+ 1.2505	
2. III.	9 950	540	1.8034	3.7480	0.804		+ 1.2505	Karmin
3. III.	9 880	880	2.4761	5.8107	0.418		+ 1.2505	Ab 3. III. mittags 2.5 mg aktives Eisenoxyd pro kg Körpergew.

Table V. Dog no. 1. Main period

1= date; 2= 24-hour urine amount; 3= bddy weight;
4= urine C; 5= urine N; 6= C:N (i.e. : in German is division sign ./.) 7= dry excrement amount per period;
8= daily N balance in the period average; 9= remarks
10= food see table 1. The food was eaten daily. Daily 2.5 mg Fe_2O_3 per kilogram. 11 = in the urine during this period there was no protein, no sugar. Daily Fe_2O_3 per kg of body weight 2.5 mg.
12= daily Fe_2O_3 per kg of body weight 2.5 mg.
13= Daily Fe_2O_3 per kilogram body weight carmin 2.5 mg.

Tabelle V. Hund 1. Hauptperiode.

1 Datum 1927	2 24-stündige Harnmenge	3 Körpergewicht g	4 Harn-C	5 Harn-N	6 C:N	7 Trockenkot insges. pro Periode g	8 Tägliche N-Bilanz im Periodendurchschnitt	9 Bemerkungen	
4. III.	700	9 500	4,1850	6,8845	0,607	123,5	+ 2,5450	Nahrung s. Tabelle I. Nahrung wird restlos aufgefressen. Täglich 2,5 mg Fe_2O_3 pro kg Körpergewicht	10
5. III.	570	10 050	2,0520	4,3442	0,473		+ 2,5450	Im Urin während der ganzen Periode kein Albumen, kein Zucker. Täglich Fe_2O_3 pro kg Körpergewicht 2,5 mg	11
6. III.	660	10 650	2,6862	4,8014	0,557		+ 2,5450	Täglich Fe_2O_3 pro kg Körpergewicht 2,5 mg	12
7. III.	650	10 250	1,9500	3,5140	0,555		+ 2,5450		
8. III.	170	10 100	0,8262	1,5606	0,529		+ 2,5450		
9. III.	450	10 200	2,0350	3,4605	0,589		+ 2,5450		
10. III.	310	10 100	2,4490	4,1035	0,596		+ 2,5450	Täglich Fe_2O_3 pro kg Körpergew. Karmin 2,5 mg	13

Table VI. Dog no. 2. pre-period
same captions as table IV,

1= Food see table II. The food was eaten daily.
2 = During the entire period no protein, no sugar in the urine.
3= carmin

Datum 1927	Körpergewicht g	24-stündige Harnmenge	Harn-C	Harn-N	C:N	Trockenkot insges. pro Periode g	Tägliche N-Bilanz im Periodendurchschnitt	Bemerkungen	
14. II.	6600	790	1,8304	5,6665	0,327	123,5	+ 0,18	Nahrung s. Tabelle II. Nahrung wird täglich aufgefressen	1
15. II.	6700	800	1,7081	5,3884	0,318		+ 0,18	Während der ganzen Periode kein Albumen, kein Zucker im Urin	2
16. II.	6600	610	1,8438	5,2710	0,353		+ 0,18		
17. II.	6670	620	1,1265	5,0212	0,244		+ 0,18		
18. II.	6610	560	1,4298	4,5733	0,314		+ 0,18		
19. II.	6670	580	1,6190	5,1692	0,317		+ 0,18		
20. II.	6600	630	1,5923	4,8732	0,324		+ 0,18		
21. II.	6530	640	1,2650	4,4833	0,283		+ 0,18	Karmin	3

Table VII. Dog No. 2. Main period

(same captions as table IV)

1= food see table II. The food was eaten daily. Daily 5 mg Fe_2O_3 per kilogram (body weight).
2= No protein, no sugar in the urine during the entire period.
3= Daily 5 mg Fe_2O_3 per kilogram of body weight.
4= Daily 5 mg Fe_2O_3 per kilogram of body weight, carmin.

Datum 1927	Körpergewicht g	Zulage (Traubenzucker) g	Harn-C	Harn-N	C:N	Trockenkot pro Tag g	Tägliche Nahrung im Versuchsdurchschnitt	Bemerkungen	
22. II.	6550	650	1.4560	4.7770	0,304	66,5	+ 0,92	Nahrung s. Tabelle II. Nahrung wird täglich aufgefressen. Täglich 5 mg Fe_2O_3 pro kg	1
23. II.	6550	540	1,4074	5.42[illegible]7	0,256		+ 0,92	Während der ganzen Periode im Urin kein Albumen, kein Zucker	2
24. II.	6400	450	2.57[illegible]6	4.62[illegible]3	0,556		+ 0,92		
25. II.	6550	660	2.[illegible]416	4,8673	0,425		+ 0,92		
26. II.	6550	60[illegible]	3.7732	4.27[illegible]3	0,885		+ 0,92		
27. II.	6600	620	3.7038	4.1084	0,902		+ 0,92	Täglich 5 mg Fe_2O_3 pro kg Körpergew.	3
28. II.	6550	650	3.1218	4.8207	0,70[illegible]		+ 0,92		
1. III.	6550	500	2.7[illegible]0	4.2[illegible]4	0,[illegible]87		+ 0,92		
2. III.	6[illegible]0	650	2.7715	4.2[illegible]5	0,855		+ 0,92		
3. III.	6550	770	2.7483	3,[illegible]836	0,[illegible]4[illegible]		+ 0,92		
4. III.	6480	850	3.2072	5.3361	0,601		+ 0,92	Täglich 5 mg Fe_2O_3 pro kg Körpergew. Karmin	4

Table VIIIa. Dog No. 2. After-period I.

(same captions as table IV)

1= food see table II. The food was eaten completely.
No protein, no sugar in the urine during the period.

Datum 1927	Körpergewicht g	Zulage (Traubenzucker) g	Harn-C	Harn-N	C:N	Trockenkot pro Tag (Nachperiode I+) g	Tägliche Nahrung im Versuchsdurchschnitt	Bemerkungen	
5. III.	6550	640	3.[illegible]046	5,0391	0,605	208,5	+ 0,25	Nahrung s. Tab. II. Wird ganz aufgefressen. Während der Periode im Urin kein Albumen, kein Zucker	1.
6. III.	6550	70[illegible]	3.8181	5.1240	0,745		+ 0,25		
7. III.	6540	80[illegible]	2.3391	4,8084	0,528		+ 0,25		
8. III.	6510	6[illegible]0	3.3590	4,5729	0,734		+ 0,25		
9. III.	6550	500	2.6006	4,1136	0,646		+ 0,25		
10. III.	6[illegible]0	720	3.6864	5.1060	0,721		+ 0,25		
11. III.	6550	820	2.8072	5,0009	0,525		+ 0,25		
12. III.	6570	[illegible]0	3.5280	4,7746	0,738		+ 0,25		
13. III.	6550	5[illegible]0	3.1878	5,0861	0,619		+ 0,25		
14. III.	65[illegible]	660	2.9438	4,9048	0,600		+ 0,25		
15. III.	6[illegible]0	7[illegible]0	2.9496	4,8112	0,[illegible]84		+ 0,25		
16. III.	64[illegible]	[illegible]0	3.6426	4.7626	0,756		+ 0,25		
17. III.	6[illegible]0	[illegible]0	2.7923	4.9931	0,559		+ 0,25		
18. III.	6[illegible]5	720	2.5385	4,7201	0,601		+ 0,25		
19. III.	6[illegible]0	580	3,8205	5,4038	0,706		+ 0,25		

Table VIIIb. Dog no. 2. After-period 2.

(same captions as table IV)

1 = food see Table II. The food was eaten daily.
2 = During the period no protein, no sugar in the urine.

Datum 1927	Körper-gewicht g	Harnmenge ccm	Harn-C	Harn-N	C : N	Trockenkot-menge Periode 1+2 g	Nüchtern-Harnstoff-Durchschnitt	Bemerkungen
20. III.	6380	760	2,0702	5.1109	0,381	203.5	+ 0,25	Nahrung s. Tabelle II. Futter wird tägl. aufgenommen. 1
21. III.	6350	520	2.0886	5.1977	0,573		+ 0,25	Während der Periode kein Albumen, kein Zucker im Urin 2
22. III.	6350	600	3,1772	7.2947	0.441		+ 0,25	
23. III.	6300	550	3,0421	6.8479	0.444		+ 0,25	
24. III.	6450	620	3.0634	4.5955	0.666		+ 0,25	
25. III.	6700	620	3.2014	5.8249	0.563		+ 0,25	
26. III.	6800	500	3.0916	4.6087	0.670		+ 0,25	
27. III.	6800	420	2,0019	3,6990	0,554		+ 0,25	
28. III.	6200	550	2.2400	5.4612	0.777		+ 0,25	
29. III.	6270	600	2.6244	6,2111	0.422		+ 0,25	
30. III.	6250	520	3.0680	5,8401	0.502		+ 0,25	
31. III.	6270	700	2.5091	4.8364	0,518		+ 0,25	

Table IX. Dgg No. 1

1= C urine; 2= N urine; 3= C divided by N; 5= N excrement
6= food N; 7= protein oxidation ; 8= N balance
9= average per day in the period
10= pre-period; 11= main period

1 Harn-C	2 Harn-N	3 C : N	4 Kot-C	Kot-N	5 Nahrungs-N	7 Eiweiß-oxydation	8 N-Bilanz
2	3			4	5	7	8
9 im Durchschnitt pro Tag in der Periode							
10 Vorperiode							
2.0125	5.4403	0.427	2.0	0.8	7.45+8	81,2	+ 1,21.3
11 Hauptperiode							
2.0557	4.1405	0.57.	2.0	57?	7.45+8	60,1	+ 2.5403

Table X. Dog No. 2

(same captions as Table IX).

1 = after-peridd 1; 2 = after-period 2.

Harn-C g	Harn-N g	C : N	Kot-C	Kot-N	Nahrungs-N g	Elektive oxydation %	N-Bilanz
im Durchschnitt pro Tag in der Periode							
			Vorperiode.				
1,5856	5,0609	0,312	1,60	0,577	5,83	96,5	+ 0,15
			Hauptperiode.				
2,7289	4,6171	0,591	1,04	0,261	5,83	83,5	+ 0,02
			Nachperiode 1.				
3,1982	4,8841	0,654	1,35	0,44	5,83	93	+ 0,25
			Nachperiode 2.				
3,0478	5,5537	0,545	1,35	0,44	5,83	93	+ 0,25

PYLORIC STENOSIS AND FIBROUS STRICTURE OF THE STOMACH DUE TO FERROUS SULPHATE POISONING

BY

F. G. M. ROSS, M.B., B.Ch., D.M.R.D.
Formerly Senior Registrar, Hammersmith Hospital, W.12

During recent years several accounts of the effects of poisoning from compound ferrous sulphate tablets have been published, notably by Forbes (1947), Thomson (1947, 1950), and Spencer (1951). There has been an increasing awareness of the danger if these tablets are accidentally swallowed by children. The following case is thought worthy of record, as it exhibits certain features not noted in the cases so far reported.

Case Report

A previously healthy boy, aged 17 months, swallowed between six and twelve tablets of a proprietary preparation of compound ferrous sulphate at about 2 p.m. on July 21 1951. He vomited shortly afterwards, and this was followed by haematemesis. On admission to hospital an hour later he was found to be restless and collapsed. Gastric lavage was performed and anti-shock treatment instituted. He continued to vomit small quantities of bright-red blood all that day. Next day he was quieter and vomited some dark-brown fluid only once, at 11 a.m. No further haematemesis occurred. He was discharged on August 3, by which time he was vomiting only occasionally and his stools were normal in colour. His weight at this time was 19 lb. 4 oz. (8,730 g.).

FIG. 1.—Barium meal August 28, 1951. Stricture of body of stomach.

After discharge from hospital he failed to improve. The vomiting gradually increased in severity, and he was re-admitted to the same hospital on August 14. On examination he was found to be dehydrated and very restless. He weighed 17 lb. 12 oz. (8,050 g.). On August 17 and 20 he vomited large quantities of curds. On August 25 his weight was 16 lb. 12 oz. (7,600 g.). A barium meal examination done on August 28 was reported to show a stricture of the body of the stomach, through which the barium passed slowly, and some oesophageal dilatation (Fig. 1).

On August 31 he was transferred to Hammersmith Hospital. He was found to be a grossly wasted and dehydrated child, with depressed fontanelle; otherwise physical examination was negative. Intravenous saline and dextrose were given, and feeds by mouth of 1 oz. (28 ml.) of milk with casydrol. He vomited five times on September 1, and seemed to have abdominal pain on taking feeds. Next day he was better and less dehydrated. It was thought that there was probably an obstruction at the lower end of the oesophagus. A gastrostomy was therefore performed on September 4, and from then on he was fed by this route. During the next week vomiting still occurred, but consisted mostly of mucus. A chest radiograph on September 11 showed the lungs to be clear. Next day stomach aspiration yielded 13 oz. (368 ml.) undigested milk and mucus

(1) September 13 x-ray examination showed the tip of the gastrostomy tube in the oesophagus and some air in the bowel. A barium meal was given by mouth. The oesophagus showed some dilatation, but no obstruction was seen at the lower end. The stomach contained excess test-fluid. A sharply demarcated constricting band was seen passing horizontally round the lower part of the body of the stomach. Distal to this the pyloric antrum was seen to be narrowed and its outline was slightly irregular. The barium shadow ended distally in a smooth convexity directed backwards and to the right (Fig. 2). Every effort was made to identify the pylorus and duodenum. However, this was not possible. No peristalsis was observed in the stomach. The gastric mobility appeared normal. At four hours no barium had left the stomach, and it was decided to remove most of it via the gastrostomy tube to avoid the danger of aspiration into the lung. Re-examination next day, at approximately 20 hours, showed there was a small amount of barium in the transverse colon, and some still remaining in the stomach. The stricture was seen at this time, as the stomach was filled with air (Fig. 3). From these investigations it was thought that there was a well-defined constricting fibrous band passing round the body of the stomach horizontally. There also appeared to be fibrosis and narrowing of the stomach distal to this stricture, with almost complete stenosis in the region of the pylorus.

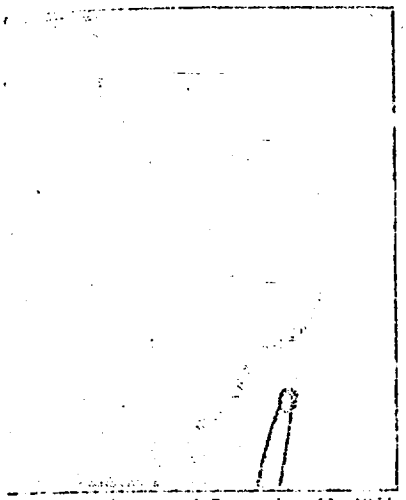

Fig. 2. Barium meal September 13, 1951. Stricture of body of stomach, narrowing of pyloric antrum, and pyloric stenosis.

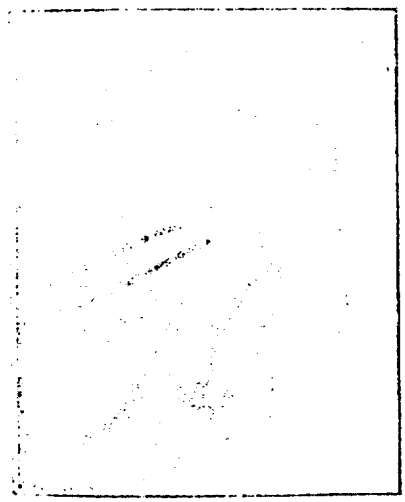

Fig. 3. Barium meal 20 hours after Fig. 2. Air content of stomach demonstrates stricture and narrowing of pyloric antrum. Small amount of barium in transverse colon.

On September 15 jejunostomy was performed, and three days later hourly feeds were started through the tube. In the next three days he gained 11 oz. (312 g.) in weight, and his general condition was somewhat improved. However, subsequent to this the child's condition gradually deteriorated, and he died on September 20.

Necropsy (Dr. Keith Simpson). Forty hours after death. Body in a poor physical state, with considerable loss of flesh and marked dehydration. Recent operations for both gastrostomy and jejunostomy completed successfully and without operative mishap but with suppurative peritonitis supervening after the second of these procedures. No residual corrosive change at the lips or in the glottis and no mechanical obstruction to the cardiac orifice. Liver showed mild toxic change only. Some excess mucus and oedema fluid was found in the bronchial tree, but no pneumonitis nor pulmonary collapse was seen. There was early extension of infection into the right pleural sac from the peritoneum. In the stomach there was remarkable thickening of the wall by scar tissue extending distally from the lower third of the body. A broad band of scar tissue contracted the pyloric antrum transversely, and thickening of the wall extended into the pyloric sphincter. In the scarred area there was distortion and narrowing of the gastric lumen.

The cause of death was acute suppurative peritonitis following jejunostomy for obstruction to the stomach by scarring due to poisoning with compound ferrous sulphate tablets.

Discussion

Very few cases of pyloric stenosis and fibrous stricture of the stomach due to the ingestion of poisons, without producing concomitant fibrous stricture of the oesophagus, can be found in the English literature, but apparently such cases are more common in foreign countries. Cases have been reported due to the following causative agents: sulphuric acid (Halstead, 1919); hydrochloric acid (Schulenburg, 1941; Joll, 1941); formaldehyde (Vinson and Harrington, 1929); tincture of iodine (Wilensky and Kaufman, 1939); and lysol (Grey Turner, quoted by Schulenburg, 1941). Very recently a case has been reported of pyloric stenosis arising in a 3-year-old boy about one month after swallowing 67 compound ferrous sulphate tablets (Crosskey, 1952).

Compound ferrous sulphate tablets consist of ferrous sulphate 3 gr. (0.2 g.), copper sulphate 1/25 gr. (2.6 mg.), and manganese sulphate 1/25 gr. (2.6 mg.), usually coated with sugar and coloured bright green. Somers (1947) and Forbes (1947) have shown that the ferrous sulphate is the lethal agent, the small amounts of copper and manganese making no difference to the toxicity of the tablets. Forbes (1947) and Spencer (1951) have independently suggested that the ferrous sulphate is converted in the stomach into ferric chloride, the latter producing the main local lesions.

Twenty cases of poisoning with compound ferrous sulphate tablets in children have been reported in the literature so far. Of these, 10 (50%) have recovered, all apparently remaining symptom-free after discharge from hospital, and 9 (45%) have died within two and a half days. One case (5%) recovered, but subsequently developed pyloric stenosis. Nearly all these patients vomited after taking the tablets, but 66.7% of those that died had haematemesis or blood in the stomach washings, compared with only 30% of those that recovered without sequelae.

Experimental work by Somers (1947) and Forbes (1947) in animals has demonstrated that the gastric changes are roughly proportional to the size of the dose for each species. In children the gastric appearances at necropsy have been consistent and similar to those found in the experimental animal. In children there is an area of haemorrhagic gastritis with oedema (Prain, 1949) situated in the middle third of the stomach near the greater-curve side and involving both walls. A less degree of similar reaction extends over the rest of the stomach. Microscopy shows oedema of the submucosa, necrosis of the mucous membrane, vascular thrombosis, and impregnation of the mucosa and vessel walls with iron. Forbes (1947) and Spencer (1951) emphasize the haemorrhages that occur into the stomach wall.

It is unfortunate that the stomach in the present case was not examined histologically. It is evident, however, from the accounts given of similar cases and experimental work in animals that the process of repair following the intense haemorrhagic gastritis, if given time, would lead to fibrous contracture of the pyloric antrum and pyloric stenosis. This fibrosis may also be increased by superficial peptic ulceration and subsequent scarring following the shedding of the gastric mucosa. In fact, the development of the fibrous reaction in the submucosa and muscular coat of the pylorus was noted in the biopsy specimen in Crosskey's case (1952).

In the present case the rapid development of the fibrous stricture of the pyloric antrum and the pyloric stenosis can be seen by comparison of the two barium-meal examinations done 16 days apart. This clearly excludes a congenital abnormality.

Summary

A case of pyloric stenosis and fibrous stricture of the pyloric antrum developing in under two months in a child after swallowing compound ferrous sulphate tablets is described. Death was due to acute suppurative peritonitis following jejunostomy.

The literature on ferrous sulphate poisoning and fibrous stricture of the stomach due to the ingestion of corrosive poisons is briefly reviewed.

My thanks are due to the physicians and surgeons under whose care the patient was at Hammersmith and Margate Hospitals for permission to publish the case, to Dr. J. Duncan White and Dr. J. P. Thierens for permission to use the radiographs, to Dr. Keith Simpson for his necropsy report, and to Mr. Basden for the reproductions of the radiographs.

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THE USE OF THE DOG FOR STUDIES ON IRON AVAILABILITY[1]

W. R. RUEGAMER, L. MICHAUD, E. B. HART
AND C. A. ELVEHJEM
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

TWO FIGURES

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Dietary studies on the relation between the antianemic potency of various foods and their iron content when fed in the presence of adequate copper, have been made by numerous workers. The rat has been used almost exclusively in these investigations and consequently the applicability of these results to human nutrition may be open to some question. Since clinical investigations of this type are difficult, it follows that the work should be repeated with other species.

The dog was chosen for this study, and attempts were made to determine the availability of iron in several foods for the dog, and to determine the differences if any that exist between these values and those reported for the rat.

METHODS

Our first problem was to determine the minimal level at which the iron and iron containing foods should be fed to produce optimal hemoglobin formation. This would prevent the accumulation of large quantities of iron in the various tissues of the animal.

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To determine this level of iron, a litter of 5 collie puppies (1–6) and later a litter of 5 spaniel puppies (7–11) were placed on experiment. As in previous iron and copper work (Maass et al., '44) a diet of raw whole milk supplemented with vitamins and minerals, was fed ad libitum. That this diet is complete for the dog was demonstrated by Potter et al. ('38) who maintained dogs for long periods of time on a whole milk ration supplemented only with vitamins A and D together with iron, copper and manganese. If the proper precautions are taken against contamination, the amount of iron supplied by the diet is found to be very low (Frost et al., '40a). However, as a safeguard, the iron content of the milk fed was determined at frequent intervals (Ruegamer et al., '45).

Iron was supplied as ferric pyrophosphate.[2] This iron salt was selected because it is stable in solution and the iron is completely available. This salt together with the other mineral supplements, was added to a small amount of milk at each morning feeding.

Blood for analysis was removed from the radial vein, all samples being collected before the morning feeding. The external jugular and later the saphenous artery were used for phlebotomy when it became necessary to render the animals anemic. From 25 to 45% of the total blood volume (calculated as 8% body weight) was removed at each bleeding without apparent injury to the animals. One animal was later sacrificed for histological study and only mild hyperplasia of the bone marrow was noted.

Records were kept of the growth, the hemoglobin levels and the amount of iron fed. From these data, the total hemoglobin in the dog, hemoglobin made, iron used and the percentage of iron utilized were calculated (Frost et al., '40a). The percentage of availability of the iron in the test material can be calculated by assuming the inorganic iron to be 100% available. It should be noted that the iron furnished by the milk was not considered in the availability calculations since this factor remained constant throughout the experiment. In all

calculations, the total blood volume was considered to be 8% of the body weight. As shown by Hahn et al. ('42), the total blood volume of the dog is maintained at a constant level independent of the state of anemia.

The amount of plasma iron was determined routinely by the improved method of Kitzes et al. ('44) in an attempt to correlate the plasma iron levels with hemoglobin formation. Moore et al. ('37) state that iron is transported as plasma iron and that the quantity present in the peripheral blood is influenced by and is a measure of the amount of iron being absorbed from the gastro-intestinal tract, the iron reserve and various other factors.

RESULTS

The positive control (dog 1) from the litter of collie puppies was given 3 mg of iron per kg of body weight per day in addition to the regular mineral supplements and showed normal growth and hemoglobin levels throughout the experimental period. The remaining 5 dogs which were placed on the experimental diet at weaning, developed a severe anemia (Hb 2.1 to 3.8 gm %) in 4 to 5 weeks. At the end of the fifth week, dog 2 was given 200 µg, dogs 3 and 4 400 µg, and dogs 5 and 6 800 µg of iron per kg of body weight per day. At the end of the first week of iron therapy, dogs 2 and 3 showed no hemoglobin response. Therefore, the level of iron for these animals was raised to 600 µg of iron per kg of body weight per day. A rapid hemoglobin response occurred, suggesting that 600 µg might be adequate for growing dogs. Dog 4 continued to make hemoglobin throughout the entire experiment even though confined to the lower level of 400 µg of iron. Dogs 5 and 6, which were kept on 800 µg, continued to make hemoglobin rapidly, showing a hemoglobin increase from 4.2 and 2.1 at the start of the experiment to 9.1 and 7.5 gm % at the end of the 6-week period. To determine whether the 600 or the 800 µg level was most efficient for hemoglobin building, the per cent utilization for the iron was calculated and the results tabulated in table 1. At the end of the 6-week period,

the iron level of dogs 2 and 3 was raised to 1000 µg per kg of body weight per day. The per cent utilization for this level may also be found in table 1.

It was found that at a level of 600 µg of iron per kg per day (dogs 2 and 3) the utilization of the iron supplied as ferric pyrophosphate for hemoglobin building was 60 to 71%. Hemoglobin curves for the animals (dogs 2 and 3) receiving 600 µg of iron per kg of body weight per day showed approximately

TABLE 1

Utilization of ferric pyrophosphate for hemoglobin building over a 6-week period.

DOG NO.	LEVEL FED	IRON USED	IRON FED	IRON
	µg/kg/day	mg	mg	%
2	600	127	212	60
2	1000	210	541	39
3	600	134	189	71
3	1000	161	474	34
4	400	127	173	74
5	800	272	379	72
6	800	166	269	61
7	600	330	483	69
9	600	254	502	51
10	600	233	358	65
11	600	234	415	56

the same slope as that for the positive control (dog 1), receiving 3 mg of iron per kg per day (fig. 1). Levels of 800 µg (dogs 5 and 6) gave utilization of iron varying from 61 to 72% and levels of 1000 µg (dogs 2 and 3) caused the utilization to drop to 39 to 34% as would be expected. Dog 4 averaged 74% utilization on a level of 400 µg even though dog 3 failed to make hemoglobin at this level. Therefore, it can be concluded that the level of iron necessary for optimal hemoglobin building falls between 600 and 800 µg per kg of body weight per day.

From the data as plotted in figure 1, there appears to be a definite relationship between the intake of iron and the amount of iron in the plasma. When iron in excess of that

required for optimal hemoglobin formation is fed, the amount of iron in the plasma is increased. Thus when levels exceeding 600 μg of iron are fed, "normal" plasma iron values of 100–200 μg of iron per 100 ml plasma are found, and when levels below 600 μg of iron are fed, the plasma iron level drops to and sometimes below the critical level of 50 μg of iron per 100 ml of plasma. Since hemoglobin formation is greatly reduced when less than 600 μg of iron are fed, it may be concluded

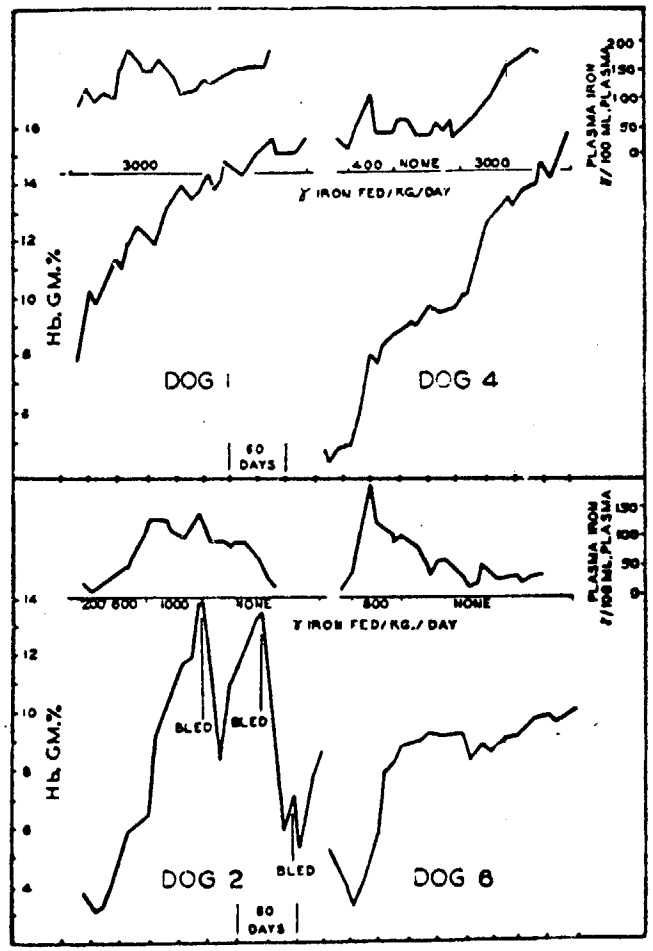

Fig. 1 Hemoglobin and plasma iron curves for dogs receiving different levels

that hemoglobin production is limited when the plasma iron level drops below 50 μg per 100 ml of plasma.

To verify our original conclusions, dogs 7–11 with the exception of dog 8 which served as a negative control, were given 600 μg of iron per kg of body weight per day after having been made anemic on a whole milk ration. At the end of 6 weeks, it was found that the animals were utilizing between 51 and 68% of the iron for hemoglobin building (table 1). These results agree with those obtained with the first litter of dogs, considering the breed difference and extent of individual variation. Therefore, it was decided to feed several biological materials at a level such that they would furnish 600 μg of iron per kg of body weight per day.

Wheat bran was selected as the first material to be fed because of its relatively high iron content, and palatability. For the experiment, the same 5 spaniel dogs were used. They were rendered anemic (hemoglobin 5–7 gm %) by phlebotomy. Between 30 and 40% of the total blood volume was removed at a single bleeding so as to render the animals anemic as quickly as possible. Once depleted of their iron reserves as evidenced by a plateau in the hemoglobin curves, dog 7 was chosen as a positive control and received 600 μg of iron as pyrophosphate per kg of body weight per day. Dog 8 served as a negative control and received no iron while dogs 9, 10 and 11 each received iron at a level of 600 μg per kg of body weight per day in the form of wheat bran.

The iron content of the bran was determined by ashing a 1 gm sample, dissolving the ash in dilute hydrochloric acid (1 part hydrochloric acid to 1 part water) and neutralizing and buffering the solution to a pH of 4.58. An aliquot was withdrawn, a reducing agent (thioglycolic acid) was added, and the amount of iron present was determined in an Evelyn Colorimeter with the addition of α-α-dipyridyl. A total of 16 samples of wheat bran were analyzed and the iron content found to be 11.5 mg 100 gm of bran, with a deviation of ± 2%. Since the animals weighed between 13 and 17 kg, between 70 and 100 gm of bran were fed daily to each dog. This supple-

ment was mixed with a little milk at each morning feeding, and the dogs were watched closely until the mixture was consumed.

At the end of the 6-week period, it was found that the utilization of iron as supplied by the bran and as fed as pyrophosphate, were approximately the same (table 2). Likewise, the slopes of the hemoglobin curves for dogs receiving bran (9, 10 and 11) were equivalent to the slope of the curve for

TABLE 2

Iron utilization for dogs receiving bran, spinach or ferric pyrophosphate as a source of iron over a 6-week period.

SOURCE	DOG NO.	LEVEL FED	IRON USED	IRON FED	IRON UTILIZED
		µg/kg/day	*mg*	*mg*	*%*
Bran	7[1]	600	209	406	51
	9	600	259	385	68
	10	600	209	330	69
	11	600	259	429	60
Spinach	7	600	30	266	11
	9	600	51	274	18
	10	600	41	216	19
	11[1]	600	134	260	51
Ferric pyrophosphate	7	600	203	516	59
	8	600	295	458	65
	9	600	248	365	68
	10	600	214	409	52
	11	600	151	337	45

[1] Control dogs receiving iron as ferric pyrophosphate.

the dog receiving iron (fig. 2). During this time, the negative control (dog 8) failed to make any significant amount of hemoglobin.

The supplements were discontinued and the dogs were rendered anemic by phlebotomy and maintained at this level for 3 or 4 weeks to make certain that the iron stores were depleted. Spinach was then fed as a supplement. The spinach was washed very carefully and dried at 46°C. by passing a stream of hot air over the material. The dried spinach was ground and the iron content determined [illegible] in the range of 4[illegible]

bran. 20.7 mg of iron per 100 gm of dried material were found, thus necessitating the feeding of approximately 40–50 gm of spinach to each dog daily. However, since the spinach proved to be unpalatable, it became necessary to mix the spinach with a small amount of milk at each feeding and give it by stomach tube. In this experiment, dog 11 received inorganic iron and served as the positive control and dogs 7,

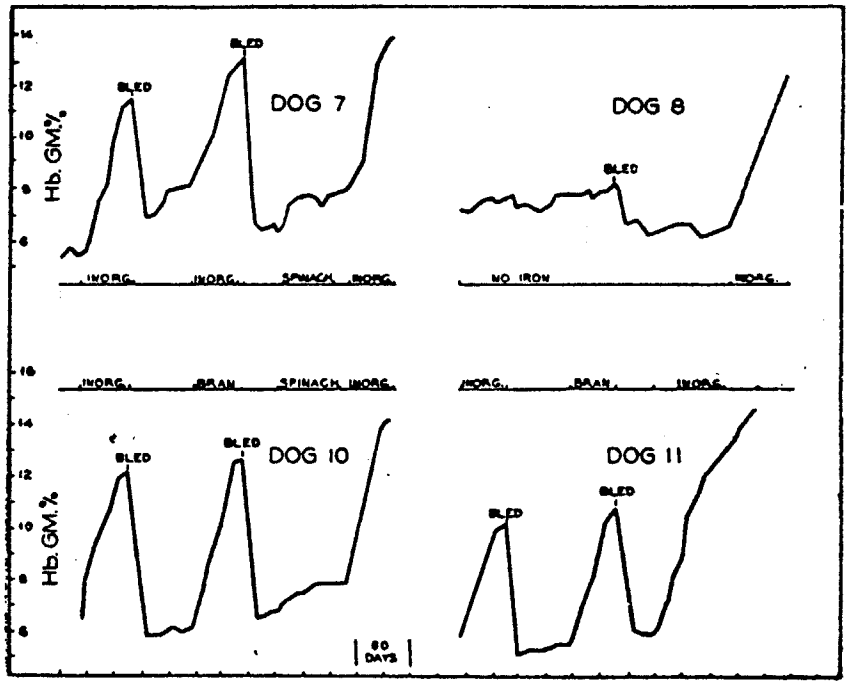

Fig. 2 Hemoglobin curves for dogs receiving iron as wheat bran, spinach and pyrophosphate.

9 and 10 received the spinach. Dog 8 served as the negative control as before. At the end of a 6-week period it was found that the spinach dogs (7, 9 and 10) utilized only 10 to 20% of the iron in the spinach for hemoglobin building, whereas the positive control (dog 11) utilized 50% (table 2). As can be seen from figure 2, there is also a marked difference in the slopes of the curves showing hemoglobin regeneration

To determine if phlebotomy had had any effect on the blood building mechanism, all animals were given iron as pyrophosphate at a level of 600 µg per kg of body weight per day. As before, the animals utilized between 52 and 68% of the iron for hematopoiesis (table 2).

DISCUSSION

The results of this experiment indicate that levels of 600 to 800 µg of iron per kg of body weight per day are optimal for hemoglobin formation in the dog. Breed difference seems to have little or no effect on the iron requirement since it was possible to obtain essentially the same results in two breeds of dogs. If less than 600 µg of iron are fed, the animals will fail to make hemoglobin, and if more than 800 µg are fed, the per cent utilization will decrease. Therefore, since storage of iron in the tissues is to be avoided, the test materials were fed at a level such that 600 µg of iron were supplied per kg of body weight per day. That little storage of iron took place is shown by the hemoglobin curves (fig. 2). In each case, when rendered anemic, usually by one bleeding, the animal failed to make hemoglobin until iron supplementation was started.

The iron in bran was found to be almost completely available for hemoglobin formation. Of course, there is the possibility that additional factors supplied by the bran stimulated hemoglobin production. This seems unlikely however, since it has been demonstrated that milk will support hematopoiesis in dogs without the addition of factors other than iron, copper and manganese (Maass et al., '44; Frost et al., '40a, b).

When spinach was fed as the sole source of iron, very poor hemoglobin regeneration occurred. The per cent utilization of the spinach iron averaged between 10 and 20% as compared to 50% for the iron given as ferric pyrophosphate. As mentioned previously, the spinach was dried at 46°C. to simplify the feeding problem and this processing might possibly have had some effect on the availability of the iron, since it has

been found that refrigeration, for example, will increase the availability of the iron in spinach (Hastings et al., '41). Under the conditions of this experiment, however, we found the availability of iron in spinach to be very poor.

Little iron contamination of the basal milk ration occurred as evidenced by the failure of the negative control to produce significant amounts of hemoglobin, and by the frequent assays of milk fed. However, one animal (dog 4) did make hemoglobin on the low level of 400 µg of iron per kg of body weight per day, and thus it is possible that this animal obtained iron from outside sources either from the cage or through handling.

When the values for the availability of iron in bran and spinach as obtained with this assay method on dogs are compared to those obtained with rats, a close correlation is found. Recent work in this laboratory with the rat has shown that the iron in bran is completely available as compared to ferric pyrophosphate (unpublished data). Sherman et al. ('34) found the iron in spinach as assayed by the 2-2-bipyridine method and also the rat biological method to be only 20% available.

SUMMARY

Young growing dogs were placed on a raw whole milk ration, supplemented with vitamins, copper and manganese. When the dogs were anemic, supplements of ferric pyrophosphate at levels ranging from 200 µg to 1000 µg of iron per kg of body weight per day were supplied. A minimal level of 600 µg of iron was found to give an optimal hemoglobin response.

Plasma iron levels were followed throughout the period of iron supplementation, and it was found that when iron in excess of that required for optimal hemoglobin formation was fed, the amount of iron in the plasma increased. If suboptimal amounts of iron were fed, the plasma iron level dropped to and sometimes below the critical level of 50 µg of iron per 100 ml plasma.

Wheat bran and spinach were fed at a level to supply 600 μg of iron per kg of body weight per day, and the response compared with that obtained with ferric pyrophosphate. The iron in bran was found to be almost completely available while the iron in spinach was only 20–40% available.

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THE DETERMINATION OF IRON IN FERRIC AMMONIUM CITRATE AND IN SYRUPS CONTAINING THIS SALT

by

Luis Enrique Gaviria Salazar* and Antonio Otálora*

The object of the present study was to make a comparison of the different methods used in the determination of iron in the precedingly mentioned substances, and establishing the causes of error which interfere in the analysis, with the end of making some suggestions for avoiding them.

Samples analyzed:

Two original samples of brown ferric ammonium citrate, from the firm of Merck, with an approximate iron content of 28%.

Two samples of syrups.

Methods utilized:

1. Complexometric method with EDTA (Ethylenediamine Tetra-Acetic Acid sodium salt).
2. U.S. Pharmacopeia XII method.
3. British Pharmacopeia method, 1958 ed.

1. COMPLEXOMETRIC METHOD WITH EDTA (sodium salt)

Reagents:

EDTA solution 0.1 m.
Sulphosalycylic acid (indicator).
Concentrated hydrochloric acid.

The determination of iron was completed in the two samples of citrate and the two of syrup, strictly following the specifications prescribed by this method, and making some modifications in the same.

We made the following observations:

Taking quantities of all the samples which correspond theoretically to 20 mg of iron and titrating them with the 0.1 N solution of EDTA, we were hindered from reaching our object by the obstacle, that the color of the solutions totally impeded the observation of the end-point.

Taking samples ten times more diluted and titrating with 0.1 M EDTA, the end-point was brighter; however, due to the

* Professor in the faculties of Chemistry and Pharmacy, National University.
* Associate professor in the faculty of Pharmacy, National Universi

small quantity of reagent eroded (10ths of ml), the error in the titration was greater, and the results unacceptable from an analytical point of view.

Taking the same samples diluted ten times and titrating with 0.01 M solution of EDTA, the end-points were still notably imprecise.

Beyond this, the values obtained were very low, and in the majority of cases did not coincide with the samples duplicated.

It is conceivable that this second defect in the complexometric method for determining iron in ferric ammonium citrate lies in the fact that the iron is found in the form of a chelate and that notwithstanding acidulation with an acid mineral, in order to achieve a pH of 2 to 3, the iron complex does not split completely, and the rupture it does undergo is not immediate, but rather gradual and more or less slow, titrating in every case only the Fe^{+++} which might be present.

2. METHOD OF U.S. PHARMACOPEIA XII

As in the preceding case, two samples of citrate and two of syrup were used.

The method was followed exactly, and the following results were obtained:

	%
Sample of citrate a: Iron (Fe).....	16.67
Sample of citrate b: Iron (Fe).....	16.48
Theoretically the two samples should contain: Iron (Fe).....	28
Sample of syrup a:	
Sample of 50 ml syrup: Iron (Fe).....	0.22
Sample of 25 ml syrup: Iron (Fe).....	0.29
Sample of 10 ml syrup: Iron (Fe).....	0.36
Sample of syrup b:	
Sample of 10 ml syrup: Iron (Fe).....	0.14
Sample of 10 ml syrup: Iron (Fe).....	0.15
Theoretically the two samples should contain: Iron (Fe).....	0.42

In view of the disparity of the results, we proceeded to make the following modification in the iodometric method of the Pharmacopeia, using the sample of syrup b: two aliquote portions of 10 ml each were taken and the organic matter destroyed with 35% H_2O_2 in an alkaline medium, after hydrolysis

with concentrated hydrochloric acid. Then we determined the iron and obtained values of 0.29 and 0.31% respectively, or in other words, double the values previously obtained (0.14 and 0.15%), but in any case still much lower than the real values.

3. British Pharmacopeia Method

As this method yielded in all cases concordant results, and was followed strictly for the determination of the citrate, we will give a description of it:

Description of the method:

"Dissolve around 0.5 gms of ferric ammonium citrate, weighed exactly, in 15 ml of water. Add 1 ml of concentrated sulphuric acid and heat until the dark brown color changes to pale yellow. Freeze at 15°, add potassium permanganate at 0.1 N drop by drop until a rose color persists for 5 seconds. Add 15 ml concentrated hydrochloric acid (1.18 d.) and 2 g potassium iodide. Leave to set for 3 minutes. Add about 60 ml water and titrate with sodium thiosulphate 0.1 N, using a solution of starch as indicator."

Results obtained: %

Sample of citrate s: Iron (Fe).....27.70
Sample of citrate b: Iron (Fe).....28.00

These values coincide with those indicated on the labels of the flasks (approximately 28% iron).

For the analysis of the syrup we followed the prededing method, but it was necessary to make some modifications in order to avoid interferences:

Description of modified method:

Take 25 ml of syrup. Add 1 ml concentrated sulphuric acid and heat for 2 minutes. Freeze at 15°, then add solution of approximately 1 N potassium permanganate in parts and stirring, until there is enough excess so that the color is clearly rosy and persists for some minutes. Destroy the excess of permanganate, adding about 0.5 g solid tartaric acid. Before the permanganate, add about 50 ml water. Finally add 15 ml concentrated hydrochloric acid and 2 g potassium iodide. Leave to set for 3 minutes and titrate with sodium thiosulphate 0.1 N, using a solution of starch as indicator.

Results obtained: %

Sample of syrup b: Iron (Fe).....0.43

This result coincided with the duplicate and with the theoretical quantity of iron contained in the syrup.

On the basis of the observations and annotations made in the course of this study, others of a more general nature can be made, related to other interferences which present themselves in the analysis of iron and which were taken from the Bulletin of the Belgian Chemical Society, described through the analysis of iron by colorometric methods, but which logically can be applied to other cases:

In a water solution iron chloride undergoes a hydrolysis which gives rise to colloidal products. The degree of hydrolysis increases with time, and because of the action of the acids, decreases only progressively. Thus equilibrium between the different constituents of the system is not established instantaneously. From this it is seen that of all the particles present in the solution only the Fe^{+++} ions intervene in the reaction.

Thus it is clearly seen that for a citrate very rich in iron, the U.S. Pharmacopeia method is not applicable, since the prescribed quantity of acid is not sufficient to avoid hydrolysis and the formation of colloidal products in the iron complex. Said method can only be applied toward the determination of iron in ferric ammonium citrate established by the same Pharmacopeia, and whose iron content is found within the following limits: 16.5 and 18.5%, or with a lower iron content. If the methods of the U.S. and British Pharmacopeia are compared, it is observed that the former prescribes acidulation with 5 ml of concentrated hydrochloric acid, and the latter suggests making a solution first with 1 ml concentrated sulphuric acid and later with 15 ml concentrated hydrchloric acid. To this greater acidity is due the fact that for citrates very rich in iron, the British Pharmacopeia method always yields concordant results.

Consequently, and as a conclusion of this work, we recommend the method of the British Pharmacopeia as above described for the determination of iron in ferric ammonium citrate, and the same method, with the modifications noted, for the determination of iron in syrups, since in many cases the analyst disregards the derivation of the citrate and therefore its approximate iron content.

SUMMARY

A comparative study was made of the three methods used to determine iron in ferric ammonium citrate and syrups which contain this compound.

The methods selected were the following:

1. The complexometric method with EDTA (Ethylenediamine Tetra-Acetic Acid sodium salt).
2. The U.S. Pharmacopeia method.
3. The British Pharmacopeia method.

The following conclusions were arrived at:

The complexometric method with EDTA cannot be applied in this case, since it yields very low results due to two interferences: the impossibility of establishing the end-point of the

titration and the only partial dissolution of the iron complex when the solution is mixed with hydrochloric acid before titration. Thus in each attempt one determines only a part, major or minor, of iron, depending upon the conditions in which one works.

The U.S. Pharmacopeia method works very well when there is a question of determining the iron in the ferric ammonium citrate established by this same pharmacopeia, and the iron content of which is found within the following limits: 16.5 and 18.5%. However, for citrates with a greater amount of iron, this method is not applicable; in syrups in which a citrate is used which contains a greater amount of iron, said method is likewise inapplicable. This is due to the medium or solution used, because it lacks sufficient acidity to dissolve completely the iron complex.

The British Pharmacopeia method is the one which we recommend because, due to a greater acidity of the medium, it always yields consistent results with any ferric ammonium citrate tested. For syrups with this citrate we also recommend this method with the modifications noted.

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Quantitative Study of the Absorption of Iron Salts in Infants and Children

JEANETTE SCHULZ, M.D., Los Angeles, and NATHAN J. SMITH, M.D., Madison, Wis.

In a previous paper we reported that normal children absorb approximately 10% of iron present in milk, eggs, chicken liver, and iron-supplemented infant cereals. It was observed that children with iron-deficiency anemia absorb food iron more efficiently than do normal children.[1] Since iron supplementation of many infants' diets may be desirable, iron balance studies utilizing radioactive isotopes of iron have been continued to include the absorption of iron salts by normal and anemic children. Other investigators[2-6] using these techniques have shown that in normal and iron-deficient adults ionic iron is more readily absorbed than is food iron and that iron in the ferrous form is absorbed better than ferric iron.[2-6] Similar studies reported to date in children are limited. The work of Darby and his co-workers revealed that children 7 to 10 years of age absorb a greater percentage of a test dose of ferrous iron than do the above-mentioned normal adults. Iron absorption in the children correlated with the estimated yearly increments in body iron during growth.[7] Oettinger et al. demonstrated the ability of premature and full-term newborn infants to absorb and utilize iron in the ferrous state.[8] The present study was undertaken to determine the influence of certain liquids and the size of the iron dose on the absorption of iron salts in normal and anemic infants and children. A ferrous salt was selected because of the published reports that bivalent salts are better assimilated than trivalent ones.[11-14]

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Bank of America-Giannini Foundation Research Fellow, Department of Pediatrics, University of California [illegible] Los Angeles (Dr. Schulz); University of Wisconsin [illegible] (Dr. Smith).

Methods

Balance-study procedures utilizing Fe^{59} as ferrous sulfate for the test feeding are essentially the same as those previously published.[1] The Fe^{59} not found in quantitative stool collections was assumed to have been absorbed. Fe^{59} present in the circulating red blood cell mass two weeks after the test feeding represents the amount of the test dose of Fe^{59} utilized for erythropoiesis. Since the Fe^{59} present in hemoglobin was consistently lower (9 ± 4%) than the amount of Fe^{59} not found in the feces, hemoglobin-Fe^{59} figures in this study are not considered representative of the total amount of Fe^{59} absorbed.

Tracer doses of Fe^{59} sulfate used in the experiments were given in a commonly used pharmaceutical preparation* in addition to the nonradioactive $FeSO_4$ in the same preparation. One cubic centimeter of this mixture contains 25.0 mg. of iron. When a single test dose was given, it was administered one to two hours before lunch and at least two hours after the ingestion of other food (breakfast). The four divided test doses were given one hour before each meal and three hours after the evening meal. Children on the twice-daily test schedule received iron one hour before the noon and evening meals. Normal subjects were healthy children, aged 9 months to 5 years, whose hemoglobin, red blood cell indices, and serum iron were within the normal range for age. Current family and past histories were negative for severe illnesses or hematologic disorders. Dietary intake of these children was considered adequate. Balance studies were completed in the home, and usual feeding regimens were not altered. Children with iron-deficiency anemia were studied during their stay on the pediatric wards of the U. C. L. A. Medical Center. Diagnostic features in these patients were typical: hypochromic microcytic anemia, hypoferremia, hypersideremia, and elevated

* Fer-in-Sol, Mead Johnson & Company, Evansville, Ind.

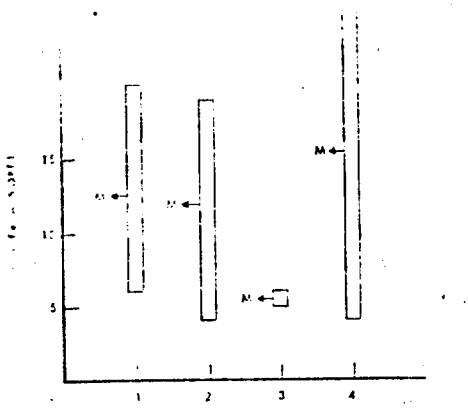

Fig. 1.—Absorption of $FeSO_4$ given two hours before meals to normal children. Group 1: 0.6 cc. t.i.d. (4×12 mg. Fe); Group 2: 1.2 cc. b.i.d. (2×30 mg. Fe); Group 3: 2.4 cc. o.d. (66 mg. Fe); Group 4: 1.2 cc. o.d. (30 mg. Fe). Bar shows range in six children; *M*, mean. There were only two children in Group 4.

free erythrocyte protoporphyrin. All responded well to therapy with ferrous sulfate after the balance studies were completed.

Results

Since the incidence of most severe iron-deficiency in pediatric patients is highest in the 6-month-old to 2-year-old group,[9,10] doses of elemental iron usually given to infants were used in the balance experiments. The first three groups of children received a total of 60.0 mg. of Fe (2.4 cc. of the test solution). The tracer doses of radioactive Fe^{59} did not add significant amounts of elemental iron to that usually present in the preparation used. Figure 1 shows the results of the balance studies in the three groups of children. Groups 1, 2, and 3 were composed of six children each, and the ages in each group ranged from 9 to 12 months to 4 or 5 years. No significant differences in the amount of iron absorbed (range 12% and 12%) were demonstrated between Groups 1 and 2, i. e., between the same total daily dose of $FeSO_4$ given in three or two daily doses. Group 3 provided good evidence that 1.2 cc. of [illegible] is the minimum amount [illegible]

children. Of the six children in the group given 2.4 cc. of Fer-in-Sol (60.0 mg. Fe) in a single dose, balance studies were completed in only two children, aged 28 months and 5 years, respectively, and only 6% and 5%, respectively, of the iron was absorbed. Two of the remaining children refused to finish the test feeding, and the other two took it well but vomited a short time later. The fourth group of six children was given a single dose of 1.2 cc. of the ferrous sulfate solution to determine if a single dose was better absorbed than two such doses in one day. More iron was absorbed from the single dose (4% to 27%), but the mean for the group (15.5%) was only slightly higher. The one child who absorbed 27% of the iron was 9 months of age, and 25% of the administered iron was used for erythropoiesis within the two-week period following the test feeding. Hematologic values in this infant were at low normal levels for his age: hemoglobin, 10.5 gm/100 ml.; mean corpuscular volume, 71 cu.μ; mean corpuscular hemoglobin, 23$\mu\mu$g.; serum iron, 64μg/100 ml. These values and the per cent of iron absorbed are perhaps a reflection of the relative iron deficiency in this apparently normal infant. Since a single

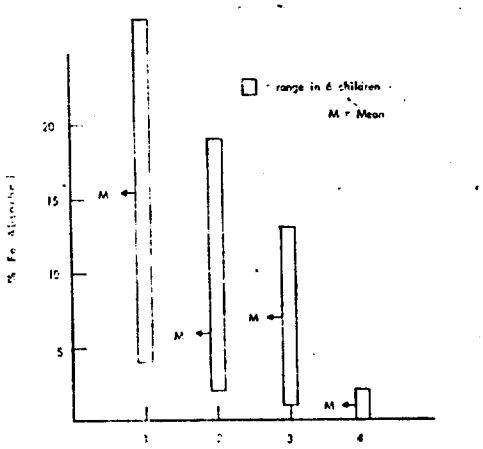

Fig. 2.—Absorption of $FeSO_4$ in normal fasting children. Group 1: 30 mg. Fe alone; Group 2: 30 mg. Fe in 2.5 cc. milk; Group 3: 30 mg. Fe in 100 cc. orange juice; [illegible] mg. Fe [illegible]; Group 4: [illegible] 1.2 cc. [illegible] 30 mg. Fe [illegible] subjects.

dose of 30 mg ... from ... [illegible] ... iron ... a day, the ... iron ... several doses of this magnitude are administered.

Figure 2 compares the quantity of iron absorbed by children given 30 mg. of Fe^{++} with the absorption when the same amount was given with milk or orange juice. The first group of six children is the same as Group 4 in Figure 1 and is included here for comparison. It is evident that 180 cc. (6 oz.) of milk exerts an inhibitory influence on the absorption of the ferrous Fe^{59}, but it seems that any increase in the volume of the test feeding has a like effect. It is unlikely that orange juice would exert any effect other than perhaps some increase in the efficiency of iron absorption. However, in the third group of six children, aged 12 months to 5 years, no such effect was observed. Only 3% to 10% of the Fe^{59} fed to these children appeared in the circulating red cell mass. No gross differences in economic status, nutrition, or hematologic values were found in these normal children when they were compared with the other test subjects. Ascorbic acid content of samples of the fresh orange juice was determined[27] before and after the feeding, and an average value of 42 mg/100 ml. was found. Moore has shown that the quantity of ascorbic acid needed to produce an increase in iron absorption is greatly in excess of that available in the amounts of orange juice usually consumed by infants and children. These studies reaffirm this conclusion.[17]

When an attempt was made to add a solution of ascorbic acid (50 mg.) to the ferrous sulfate and milk to test iron absorption from this combination, only two children, aged 2 and 3 years, tolerated the feeding. The remaining four [illegible] refused [illegible] later. [illegible] in the [illegible] method[illegible]

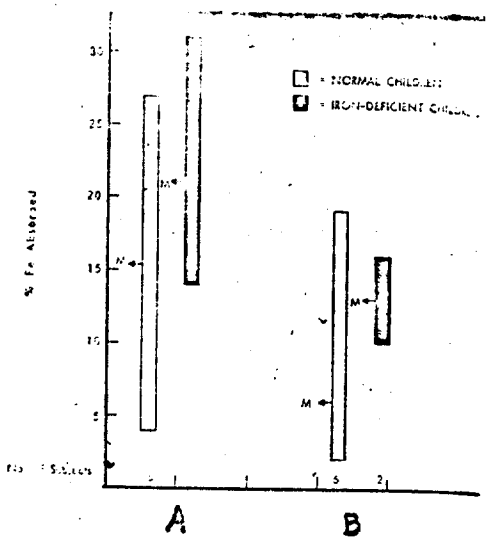

Fig. 3.—Absorption of $FeSO_4$ in iron-deficient children compared with normal children. *A*, 30 mg. $FeSO_4$ alone; *B*, 30 mg. $FeSO_4$ in 180 cc. milk.

that the above combination of ingredients was a most unpalatable mixture, which stained teeth and mouth black and resulted in nausea and vomiting in three very cooperative children. Figure 2 (Group 4) shows that very little of the Fe^{59} from this mixture was absorbed by the two children whose balance studies were completed.

Figure 3 shows that children with iron-deficiency anemia as well as normal children absorb less of a therapeutic dose of $FeSO_4$ given with milk than they do when the iron salt is given alone. When the tagged ferrous sulfate was given alone, a proportionately greater quantity of the iron was also incorporated into hemoglobin by the anemic subjects (10% to 27%). The differences between the absorption of iron, whether it be naturally occurring food iron[1] or iron salts, by normal children and children with iron-deficiency anemia are statistically significant.

Repeated balance studies with ferrous sulfate were conducted on some of the subjects in whom food-iron absorption had been determined earlier.[1] The Table illustrates the results of the two balance studies in each subject. Because of the small number of subjects, the variations in age, type, and

	Age, Mo.	Feeding	Fe in Feeding, Mg.	% Fe Absorbed
Children				
1	8	Egg	1.2	17
	13	Milk	0.09	11
2	20	Egg	1.2	17
	34	Milk	0.09	11
3	45	Egg	1.2	4
	50	Milk	0.09	7
4	16	Egg	1.2	20
	25	$FeSO_4$ + water	48.0	7
5	6	Cereal (Fe)	3.0	6
	18	$FeSO_4$ + milk	24.0	19
6	18	Cereal (Fe)	3.0	6
	30	$FeSO_4$ + milk	24.0	2
7	17	Cereal (Fe)	3.0	6
	29	$FeSO_4$ + water	24.0	13
Adult males (aged 23–25 yr.)				
1		Egg	1.2	2
		$FeSO_4$	100.0	4
2		Egg	1.2	4
		Fe pyrophosphate	360.0	1
3		Egg	1.2	4
		$FeSO_4$	100.0	2

quantity of iron administered and the bulk of the feedings, no conclusions can be made about this group of experimental subjects. Some children absorbed iron salts better than food iron from a single test feeding. Some absorbed more iron at an earlier age than several months later. Other studies in adults have shown that they can be expected to absorb iron salts more efficiently than iron from food.[5,15] The normal male adults used for repeated control studies in our laboratory did not absorb more iron salts than naturally occurring egg iron when each was given after an overnight fast. However, the total doses of iron were not comparable.

Comment

Adequate treatment of infants and children with iron-deficiency anemia depends upon the quantity and condition of iron absorption. In optimal circumstances, about one-sixth of a 30 mg. dose of ferrous sulfate may be absorbed. If three such doses are given daily, about 15 mg. of iron might be absorbed daily. Since in actual practice conditions for absorption are often less than ideal and infants are frequently not given the prescribed dose, more than the calculated amount of iron needed will probably have to be prescribed before therapy is [illegible].

[illegible] has a blood volume of 700 cc. and has a hemoglobin value of 6 gm. per 100 ml., his total hemoglobin iron is about 143 mg. (3.4 mg. Fe per gram of hemoglobin). If nonhemoglobin iron totals 60 mg. (6 mg. per kilogram, perhaps too high in this instance), the infant's total body iron is about 200 mg., since storage iron is probably depleted. This child may have been born with a deficit of 100 mg. of body iron, or he may have lost it by hemorrhage peri- or postnatally. The total body iron content of some newborn infants has been shown to be about 80 mg. per kilogram.[16] The iron requirements in the first 18 months of life have been estimated to be about 200 mg.[1,17] Earlier studies with milk iron and the present investigation make it unlikely that more than about 16% of the iron in milk is absorbed, even by the iron-depleted infant.[12] Our hypothetical infant would therefore have absorbed only about 40 mg. of iron from milk, leaving a total body deficit of an estimated 240 mg. of iron to be supplied from exogenous sources. As mentioned above, 12 mg. of ferrous iron might be absorbed daily. Theoretically, this infant's iron deficiency could be corrected in 20 days by giving a total daily dose of 72 mg. of iron in three or four divided doses. For the reasons alluded to previously, therapeutic doses of this magnitude would necessarily be required for much longer than 20 days to achieve the desired therapeutic result. We have frequently seen relapses of iron-deficiency anemia when such doses of iron were discontinued after a month's treatment, when the hemoglobin level had returned to normal and no blood loss was demonstrable. Storage and tissue iron, as well as hemoglobin iron, must be replenished before therapy is terminated.[23] That body iron other than hemoglobin iron is deficient in these patients is evident from many studies.[18-20] It has been shown that iron-containing enzymes are also reduced in iron-deficient animals.[21,22]

Robinson[25] recently presented [illegible] illustrations of the effects of long-[illegible]

iron deficiency ... [illegible] ... emphasizing the fact that depletion of tissue iron as well as decrease in hemoglobin iron and consequent anemia are detrimental to optimum well-being. Brokow and others found that normal infants' height, weight, and muscle tone were improved when iron-containing foods were added to their diets, though hemoglobin values did not rise significantly.[22] Since no investigations have been published documenting the desirability of increasing the normal hematologic values in infants from, e. g., 11 to 12 gm/100 ml., indiscriminate supplementation of "normal" infants' diets is not recommended. Good evidence exists that "iron-loaded" persons may absorb as much orally administered iron or more than do normal subjects.[5,24] Therapeutic iron is indicated only if specific evidence of iron deficiency exists. The prescription of "shotgun" hematinics for anemia, real or supposed, in infants and children is to be abhorred.

Summary

Balance studies utilizing a radioiron salt (ferrous sulfate) were made in 34 normal and 5 iron-deficient infants and children. The largest single dose of ferrous iron tolerated and absorbed well was 30 mg.

Twelve to fifteen per cent of the 30 mg. doses of Fe^{++} given once or twice a day were absorbed by normal children.

One hundred eighty cubic centimeters of milk or one hundred cubic centimeters of orange juice given with the iron salt resulted in less absorption of iron than when the ferrous sulfate alone was given.

Iron-deficient infants absorb more ferrous iron than do normal infants.

A quantitative approach to the correction of iron-depleted states in infants is presented.

University Hospitals, 1500 University Ave. (6) (Dr. Smith).

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Powdered Iron from 1681 to 1968†

M. N. Shanas, B.Sc., and E. M. Boyd, M.D.

Department of Pharmacology
Queen's University
Kingston, Ontario, Canada

Recent studies in this laboratory [1,2] have confirmed that medicinal iron powder is readily absorbed from the gastrointestinal tract and in addition have demonstrated that toxic and lethal doses are much higher than those of other iron preparations. Iron powder would appear, therefore, to be the iron preparation of choice in the therapy of iron deficiency anemia. Elemental iron was originally used by Sydenham in the treatment of anemia in 1681 [3] and our results suggest that we should return to the form of iron that he employed. The purpose of this paper is to review the history of medicinal iron powder and our studies on the low toxicity of this preparation of iron.

The Hindus used a preparation of iron called Lauha Bhasma [4] which was prepared by roasting sheets of iron followed by maceration with oil, whey, vinegar, cow's urine, and milk. The Greeks associated iron with Mars, the God of War, believing that he had imparted his strength to it. Swords which had been used in battle and which had drawn blood were allowed to rust under water and this water was then administered to the weak. Celcus (circa 25 A.D.) administered water, in which red hot irons had been drenched,

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to patients with what is now termed splenomegalic anemia [5]. He had noted that animals drinking the water around blacksmiths' shops had abnormally small spleens [4]. Celcus' treatment of iron would yield colloidal ferric hydroxide, which Meulengracht [6] described under the name of 'Idozan' as an efficient hematinic.

Iron continued to be used in a more or less symbolic manner for the next 1500 years. In 1554 Lange described chlorosis [5] which is now known as hypochromic anemia. The causes of chlorosis were subsequently debated over many years [7,8] and factors proposed included tight stays and corsets, dimly lit dwellings, poorly ventilated rooms, lovesickness, onset of menses, food of poor quality, masterbation, and excessive coitus.

In 1681 Thomas Sydenham [3], in writing of chlorosis, listed many symptoms but suggested that the only cure for the condition lay in improving the blood. Greenhill [3] translated Sydenham as saying: "I comfort the blood and the animal spirit belonging to it by giving a chalybeate for 30 days running." One of Sydenham's chalybeates was prepared as follows [3]: "Take of the filings of iron, eight grains; extract of wormwood enough to make it into three pills, to be taken early in the morning, and at five in the afternoon, for the space of thirty days, drinking after each dose a draught of wormwood wine." These pills gave dramatic improvement although the exact nature of the condition they were correcting was unknown to Sydenham.

Lemery and Geoffy demonstrated in 1713 the presence of iron in blood, and in 1746 Menghini reported that blood iron levels could be increased by the feeding of iron-rich foods; this observation was confirmed by Rouelle and Bacquet in 1747 and by v. Forcke in 1779 [9]. These findings partially explained Sydenham's success in the treatment of chlorosis with his iron pills.

Blaud [8], in 1832, named iron as a specific in the treatment of chlorosis A free translation of his statement reads as follows: "Chlorosis is sometime symptomatic of either a primitive or concomitant disease, sometimes it follows another sickness, and sometimes it is idiopathic. But in every case, it causes a vicious sanguification where the coloring matter is absent and the serosity predominates; and it is no longer able to excite the organism and to exercise its regular functions. Iron should be used all the time in the treatment of chlorosis, but doctors know that their success with this treatment is uncertain, so they avoid it; but this is because they employ a feeble dose and they do not introduce it into the organism in a conveniently modified form. If the iron does not arrive in the blood in a sufficiently great quantity, it cannot give back to this fluid the principle which it has lost; and if it is not modified in a proper manner, the centers of absorption will reject

Table 2

Iron and Water Levels of Blood and Several Tissues at 48 hr after Oral Administration of Increasing Doses of Medicinal Iron Powder to Albino Rats

Dose of administered iron powder, gm/kg	Iron levels at 48 hr				Water levels at 48 hr			
	Blood	Liver	Kidney	Carcass (minus gastro-intestinal tract)	Blood	Liver	Kidney	Carcass (minus gastro-intestinal tract)
1.0	- 2.8	- 23.1	-11.5	-26.5	+ 1.4	+0.2	- 0.9	+ 2.9
3.3	+ 8.6*	- 5.3	+ 1.1	-22.9	+ 1.3	-0.9	- 1.9	+ 4.8
10	- 3.6	+159.9*	+51.9*	+57.7*	- 9.9*	+0.8	- 5.6*	+ 2.0
33	+ 7.85*	+172.7*	+57.0*	+59.9*	-17.5*	-1.1	-10.5*	- 2.3
66	+ 29.9*	+235.6*	+92.1*	+32.5*	-19.4*	-3.0*	-12.4*	-11.9*
100	+ 18.9*	+203.7*	+88.9*	+91.6*	-20.9*	-3.3*	-13.7*	-10.8*

[a]The results are expressed as mean per cent change from controls given no iron, specifically as $[(\bar{X}_i - \bar{X}_c)/\bar{X}_c] \times 100$, where $\bar{X}_i$ is the mean in iron-treated rats and $\bar{X}_c$ in controls. An asterisk indicates that $\bar{X}_i - \bar{X}_c$ was significant at P = 0.05 or less.

Table 3

Iron and Water Levels of Blood and Several Tissues at Progressive Intervals after Oral Administration to Albino Rats of a Test Dose of 66 gm/kg of Reduced Iron

Hours after administration of test dose of iron powder	Iron levels				Water levels			
	Blood	Liver	Kidney	Carcass (minus gastro-intestinal tract)	Blood	Liver	Kidney	Carcass (minus gastro-intestinal tract)
3	+ 3.9	+ 37.5*	+ 27.7*	+48.1*	- 9.1*	+1.4	- 8.4*	-0.9
7	+ 9.2*	+ 70.1*	+ 14.3	+ 7.3	-12.3*	+3.1	-10.4*	-5.8
13	+24.0*	+122.0*	+149.2*	+72.2*	-19.0*	- 2.2	-10.2*	-7.2
19	+17.0*	+106.4*	+ 58.0*	+14.6	-19.2*	- 3.4*	-10.3*	-4.6
24	+16.1*	+114.8*	+ 81.4*	+12.5	-12.8*	- 0.1	-10.6*	-5.7
48	+29.9*	+236.2*	+ 92.2*	+27.8	-17.9*	- 1.9	-12.4*	-7.2

[a]The results are expressed as mean per cent change from controls, specifically as $[(\bar{X}_i - \bar{X}_c)/\bar{X}_c] \times 100$, where $\bar{X}_i$ is the mean in the iron-treated animals and $\bar{X}_c$ in the controls. An asterisk indicates that $\bar{X}_i - \bar{X}_c$ was significant at P = 0.05 or less.

it completely out of the organism." Thus did Blaud state the principle which still governs iron therapy, namely, give large doses of iron in an absorbable form. Blaud prescribed pills which were compounded of ferrous sulfate and potassium subcarbonate and which were administered in doses equivalent to approximately 2 gm of ferrous sulfate daily.

A series of discoveries made between the years 1832 and 1895 showed that chlorosis was indeed a state of iron deficiency. The treatment of this condition suggested by Sydenham and Blaud was successful. Following the introduction of Blaud's pill, a search began for other iron-containing preparations which could be substituted for metallic iron as prescribed by Sydenham. Many of them have been introduced into the practice of medicine including ferrous sulfate, ferrous carbonate, ferrous fumarate, iron and ammonium citrate, iron dextran complex, and others. Ferrous sulfate and ferrous carbonate have been the drugs of choice, even though a number of recorded deaths and nonfatal poisonings have been attributed to these substances [5,10]. The majority of poisonings occur among young children mainly from eating candy-coated iron pills containing ferrous sulfate.

Conventional iron salts used in the treatment of anemia may cause gastrointestinal reactions characterized by gastric distress, colicky pain, and constipation or diarrhea [11]. These complaints tend to be more prominent following ferric than ferrous salts and are more disturbing when the drug is given on an empty rather than full stomach. The presence of various dietary factors tends to reduce the absorption of iron from the gastrointestinal tract [12], but iron preparations are usually prescribed to be taken at mealtime in order to minimize the unpleasant side effects.

The therapeutic use of medicinal iron powder as a hematinic is well documented. Meulengracht [6] reported that powdered iron was as efficient as iron lactate and colloidal ferric hydroxide. He used doses of iron powder as large as 10 gm/day and reported no unpleasant side effects. Bethel et al. [13] found that reduced iron was as efficient as iron and ammonium citrate. Strauss [14] reported no difference in response to therapeutic doses of medicinal iron powder, ferrous carbonate, iron and ammonium citrate, and ferrous sulfate. Alstead [15] reported the successful treatment of anemia with reduced iron. No reference could be found in the literature to the toxicity of reduced iron. It was decided, therefore, to study the acute oral toxicity of this preparation. The The word "reduced" refers to iron powder prepared by a process of chemical reduction.

Reduced iron powder was administered orally to groups of normal, nonanemic, albino rats, 10–15 animals per group, by means of an intragastric tube [1]. It was given as a suspension in distilled water and was administered in a volume of 75 ml/kg body weight. The doses ranged from 0–200 gm/kg body weight. The LD_{50} ± S.E. was found to be 98.6 ± 26.7 gm/kg

body weight. No deaths occurred in animals receiving less than 50 gm/kg body weight. The LD_{50} of reduced iron was extremely high and in Table 1 it is compared with values for the LD_{50} of other iron preparations given orally to albino rats.

Table 1
The Median Lethal Dose of Iron Given Orally to Albino Rats as Various Salts

Preparation	LD_{50},[a] gm/kg
Iron carbohydrate complex	4
Ferrous sulfate	1
Ferrous chloride	1
Ferrous gluconate	1
Ferric chloride	0.4
Ferrous fumarate	0.3
Reduced iron	100

[a]The results are expressed as elemental iron and are reduced to one significant figure.

At test doses of 100 gm iron powder/kg body weight or greater, death was due mainly to bowel obstruction and occurred within 48 hr of ingestion of the drug [2]. When the dose was between 60 and 100 gm/kg body weight, death was usually delayed to later than 60 hr after ingestion of the drug and was primarily due to an increasingly severe gastroenteritis which in turn produced dehydration, hemoconcentration, and electrolytic imbalance. The degree of gastroenteritis increased with increasing dose. At doses of reduced iron below 10 gm/kg body weight, there was no evidence of gastrointestinal irritation [2].

In a second experiment [2], groups of albino rats, 10–16 animals per group, were given reduced iron orally in doses ranging from 0–100 gm/kg body weight. The animals were sacrificed 48 hr later when the toxicity syndrome had reached a maximum. The iron content and water levels of blood. liver, kidney, and residual carcass were determined. The results of this experiment are summarized in Table 2 and indicate that significant amounts of iron were absorbed. Doses of 10 gm/kg and over produced increasing dehydration of blood and tissues.

In a third experiment [2], 105 normal, nonanemic rats were given reduced iron orally in a single dose of 66 gm/kg body weight and were then killed in groups of 12 animals at intervals of from 3-48 hr. Iron and water levels in blood, liver, kidney, and residual carcass were determined as in the previous experiment. The results of this experiment are summarized in Table 3 and indicate that iron was absorbed as early as 3 hr after administration.

These results confirm the many reports that have appeared in the literature that powdered metallic iron is absorbed from the gastrointestinal tract and, at therapeutic doses, may be expected to show none of the unpleasant side effects that are usually associated with other iron preparations. It can, therefore, be taken between meals when absorption is not depressed by the presence of certain dietary factors. The extremely high value for the maximal LD_0, namely 50 gm/kg body weight, indicates that it is a safe preparation. A child of 15 kg body weight would have to ingest approximately 4000 capsules each containing 200 mg of reduced iron in order to reach the minimal lethal dose if comparable data apply to man.

Reduced iron is not at present considered a drug of choice in the treatment of hypochromic anemia. The reason for this is not obvious. The Canadian Vademicum International of 1967 [16], lists 45 registered iron medicaments, none of which contains metallic iron. The listing of metallic iron under the name of Reduced Iron was deleted from the British Pharmacopoeia after 1932, from the United States Pharmacopeia after 1942, and from the National Formulary after 1950. These publications do, however, list many different iron salts. But, from the standpoint of efficacy and safety, medicinal iron powder, which was first prescribed in 1681, would appear to be the drug of choice in the treatment of iron deficiency anemia in 1968.

The results described in this paper are brief summaries of material covered in considerably more detail by Shanas [17].

SUMMARY

Iron was originally believed to impart strength to the human body and was taken in various forms such as drinking the water in which swords had been steeped. In 1681, Sydenham introduced a form of powdered iron in the therapy of chlorosis with dramatic results because chlorosis was an iron deficiency anemia although this was unknown to Sydenham. Powdered iron was subsequently proven to be absorbed when given by mouth and to be an effective hematinic in iron deficiency anemia. Nevertheless, the attitude persisted that powdered iron was a rather crude drug which should be replaced by more refined substitutes and the elemental powder was deleted from the British Pharmacopoeia after the edition of 1932 and from the United States Pharmacopeia after the 12th edition of 1942. Substitutes for elemental iron, including ferrous sulfate, may produce undesired side effects and occasionally have caused death. We therefore decided to study the acute oral toxicity of medicinal powdered iron and found that its LD_{50} was from 25–200 times higher than those of other iron preparations in albino rats. Death was due mainly to bowel obstruction from oral doses of the order of one-tenth of body weight which would correspond on a body weight basis, to the ingestion of 35,000 capsules, each 0.2 gm, by a man of 70 kg body weight! Toxicity-wise, Sydenham's medicinal iron powder would appear to be the drug of choice for iron deficiency anemia.

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Ferrous Sulphate Poisoning with Gross Stricture of the Stomach

To illustrate the destructive effects following ingestion of ferrous sulphate pills, and to stress further the criminal negligence in leaving such pills within the reach of small children, the following case is briefly reported.

CASE REPORT

A male child of 20 months swallowed approximately 50 "fersolate" tablets and was admitted to hospital two hours later. An unrecorded proportion of the pills was recovered by immediate gastric lavage. There was repeated and severe haematemesis. The initial collapse was treated successfully by blood transfusion, but during the next few days there was a steady deterioration in the general condition, and it was not possible to maintain nutrition adequately by mouth because of repeated vomiting. When seen seven weeks after the onset of illness the child was found to be emaciated and dehydrated.

A barium meal showed a smooth tubular stricture affecting the middle third of the stomach (see Fig.).

The child was transferred to a paediatric unit in the hope of improving his nutrition and of preparing him for operation. Despite feeding by an intragastric tube, which was passed beyond the obstruction, and by the intravenous route, there was a progressive decline, and at no time did he seem fit for surgery. A further barium meal showed progressive and irregular stricture formation. He died 16 weeks after poisoning.

The necropsy showed dense adhesions in the upper peritoneal cavity. A small pouch of healthy stomach proximally and a normal pyloric antrum were found. The intervening stomach showed gross inflammatory changes, and a narrow channel was lined with granulations, ulcerated and adherent to surrounding organs. A large chronic ulcer penetrated the liver at one point. The muscle wall was replaced by fibrous tissue. The liver and kidneys were normal. These appearances confirmed that excision of the stomach would have been almost impossible in the last weeks of the illness.

COMMENT

There have been numerous reports in recent years of the corrosive effects of ferrous sulphate poisoning. Forbes (1947) and Thomson (1947) drew attention to the hazard of leaving attractive sugar-coated pills accessible to infants and children. Further cases have been described by Prain (1949), Spencer (1951), Crosskey (1952), Ross (1953), and Elliot-Smith and Davies (1954). Forshall and Rickham (1954) have discussed fully the complication of pyloric stenosis after ingestion of ferrous sulphate pills. Stricture of the mid-portion of the stomach, as found in the present instance, is less commonly encountered, and presents a more difficult problem of management than does a localized pyloric lesion.

If a child survives the immediate prostration and toxicity due to a heavy dosage of ferrous sulphate the mucosal changes in the stomach are apt to be severe and the subsequent contracture and ulceration progressive. Repeated haematemesis is probably an indication of excessive mucosal damage. It would appear that early operation is necessary, and it is suggested that jejunostomy should be performed as soon as possible. This should be followed by early resection of the stomach unless repeated radiological examination shows rapid improvement in the local condition. These contentions are borne out by the experience of Ross (1953), who described a somewhat similar case.

Dr. C. H. Stewart-Hess referred the child to me, and I am indebted also to Miss Isabella Forshall, to whom I transferred him for further treatment, for details of the later stages of the illness.

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JOHN A. SHEPHERD, V.R.D., M.D., Ch.M., F.R.C.S.Ed.,
Surgeon, Broadgreen Hospital, Liverpool.

FERROUS SULFATE POISONING

A Case Treated With BAL

Captain Joseph Shoss, Medical Corps, United States Air Force

ONLY in recent years has excessive amounts of ferrous sulfate been brought to attention as a possible cause of fatal poisoning in young children. The possibility that iron might combine with SH groups and interfere with cellular oxidation has been suggested. It has been suggested that iron probably results in widespread interference with cell function.[1] The possible rationale of the use of British anti lewisite (BAL, 2, 3-dimercaptopropanol) in prevention of this combination is the purpose of this paper.

BAL was used in treatment by Roxburgh (1949)[2] and Thompson (1950),[3] but no claims were made of its efficacy. Edge and Somers (1948)[4] reported experimental work in mice in which BAL was used orally or intravenously, and they concluded that it increases, rather than decreases, the toxic effects of ferrous sulfate. It has not received sufficient therapeutic trial since the above reports.

A case is reported of a 14-month old white female with severe ferrous sulfate intoxication and a favorable outcome. BAL was used in this case and was felt to have possibly been of great benefit. The use of BAL is suggested for further therapeutic trials.

CASE REPORT

A 14-month-old white female infant was admitted to the George Air Force Base Hospital at approximately 11:30 A.M. on March 18, 1953. The mother found the patient shortly after she had swallowed fifty to seventy-five 5 grain ferrous sulfate tablets at approximately 9:30 A.M. (two hours previous to admission). The mother felt that the child would be all right, and therefore did nothing. At approximately [illegible] A.M. (one hour previous to admission) the child became lethargic and began vomiting. She had vomited [illegible] to fifteen times in the hour previous to admission, and was vomiting when brought continuously on arrival at the hospital. On admission, the child was somnolent, slightly dehydrated, in early shock, and her mucous membranes were cyanotic. Her condition rapidly became more critical with continuous loose green stools and a gradual elevation in temperature to 103.4° F. (R) by 4:30 A.M. (seventeen hours after admission). Her pulse was weak and ranged around 160 per minute. Chest and heart examinations were essentially negative. The abdomen was soft. The liver was palpable one fingerbreadth below the costal margin in the right mid-clavicular line and was nontender. Bubbling oxygen through venous blood in a test tube revealed no evidence of methemoglobinemia.

On admission, she was lavaged with saline until clear contents were obtained. Gastric contents were grossly bloody and no tablets were seen. Two ounces of milk were left in the stomach after the lavage. After initial hydration, she was maintained on parenteral fluids for the first forty-eight hours. She was given oxygen per tent and prophylactic penicillin. BAL was started at 2:00 P.M. (four and a half hours after ingestion). She was given approximately 4 mg. per kilogram of body weight intramuscularly every four hours for two days, and then maintained on it two times

From the Pediatric Service, United States Air Force Hospital, George Air Force Base, Calif.

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a day for five additional days. Her coagulation was poor, and the prognosis was considered to be grave.

The patient seemed to be definitely showing improvement approximately four hours after the first dose of BAL. Her pulse became stronger (132 per minute), and her general appearance improved. She became more alert. There was no further vomiting or diarrhea after 4:00 P.M. Her cyanosis was gone by 11:30 P.M. It was felt that she seemed to show improvement in the four hours following BAL administration and became more lethargic previous to the next dose.

The blood count on admission was as follows: hemoglobin, 14.75; red blood count, 4.91; white blood count, 15,850; neutrophils, 41 (2 stabs); lymphocytes, 57; monocytes, 2. The hemoglobin and white blood count showed a gradual decline to 10.4 and 8,600, respectively, on the day of discharge.

The temperature remained elevated for twenty-four hours and suddenly fell to normal at about 7:30 P.M. of the second day. It remained normal thereafter. Gradual improvement was then seen, and she was asymptomatic by the third day. She was discharged on March 27, 1953.

CONCLUSION

(1) A case of ferrous sulfate poisoning is reported in a 14-month-old infant with a favorable outcome.

(2) The use of BAL is suggested for further therapeutic trials.

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FERROUS SULFATE TOXICITY*

Report of a Fatal Case

ROBERT P. SMITH, M.D.,† CHARLES W. JONES, M.D.,‡ AND WINSTON E. COCHRAN, M.D.§

BURLINGTON, VERMONT

FERROUS sulfate is generally considered a wholly innocuous drug. It is universally prescribed and with some abandon. The bulk of the research related to it has concerned its beneficial effects in anemias. Reports of toxicity are rare and, indeed, only 1 such case is to be found in the American literature of the last sixty years. Reports of 2 fatal cases in England stimulated descriptions of similar cases, with pertinent discussion and research. It seems important, therefore, to describe the following case in considerable detail — not only to draw attention to a drug that can be harmful and even lethal but also to promote understanding of the mechanisms of toxicity and its relation to the metabolism of iron, with the hope that wider discussion and research may be stimulated in the United States.

REVIEW OF THE LITERATURE

From 1850 to 1890 several cases of toxicity from iron compounds were reported.[1-6] References were made to severe gastrointestinal irritation and general collapse, but there was only 1 autopsied case. This had a typical gross picture.

In 1931 Hurst[7] described a case of iron encephalopathy following overdose of iron and ammonium citrate. Smith and Cook,[8] in 1934, mentioned a girl who swallowed 1.7 gm. (28 gr.) of ferrous sulfate and recovered.

In 1947 Forbes[9] reported 2 fatal cases of ferrous sulfate poisoning. In 1 of these a healthy boy, aged three years and three months, ate 50 0.2-gm. (3-gr.) tablets of an iron preparation containing also 2.5 mg. (1/25 gr.) of copper sulfate and 5.0 mg. (1/12 gr.) of manganese sulfate. He had two episodes of vomiting but seemed well for forty-eight hours, when he became jaundiced and restless, dying in fifty-three hours. Autopsy showed necrosis of the gastric and intestinal mucosas, slight renal tubular degeneration and a few pulmonary hemorrhages. In the other case a one-year-old boy swallowed 30 to 35 of the same pills. He had a hematemesis in an hour and was pale and showed signs of shock, with moist, labored breathing. In spite of immediate, vigorous treatment, he died in thirty hours. Autopsy showed bronchopneumonia, gastric and intestinal necrosis and degenerative changes in the liver, kidneys and pancreas. By animal experimentation, Forbes acquitted the copper and manganese traces as the toxic agents and established the cat lethal dose of ferrous sulfate as 0.065 gm. (1 gr.) per 64 gm. of body weight. Somers[10, 11] concurred after similar experiments.

Russell[12] mentioned that a number of pregnant women on massive iron therapy complained of headache, nausea and malaise and eventually manifested a mild albuminuria.

Thomson,[13] stimulated by these articles, reported 2 and later 6 cases of his own, all in children. They showed variously early pallor, drowsiness, vomiting, abdominal pain in 1 case, hematemesis in 4, positive guaiac or similar tests in 4 and sudden collapse and death in 2. Autopsy in 1 case demonstrated patchy atelectasis and severe gastric necrosis. The second showed much less severe necrosis.

Foucar, Gordon and Kaye[14] described a twenty-six-year-old man who ingested ¼ pound of ferrous sulfate (*USP*). He entered the hospital in shock and showed cyanosis, hematemesis and colic. In spite of gastric lavage, whole-blood transfusion and oxygen, he died in three hours. Autopsy revealed pulmonary hemorrhage and edema, erosion from the esophagus to the jejunum and congestion of the remaining bowel. Because the Prussian blue reaction was positive only in the tissues in direct proximity to the ferrous sulfate, the authors concluded that the iron "was important only as the vehicle of an anion that constituted a strong acid."

CASE REPORT

A 17-month-old girl was admitted to the hospital acutely cyanotic and unresponsive after ingestion of many ferrous sulfate tablets. The past history showed that she had been born after a normal delivery and that her health had been excellent until the evening of admission.

At 6 p.m. she was discovered to have ingested a handful of 0.3 gm. (5-gr.) ferrous sulfate tablets from a container she had found in the pocket of a coat hanging on a doorknob. She was apparently well until 10:00 p.m., when vomiting, followed by diarrhea, developed. At 1:00 a.m., because she was severely cyanotic, with vomiting and diarrhea, the family called a physician, who rushed the patient immediately to the hospital. In the vomitus 3 tablets, which had most of the enteric coating dissolved away and an estimated ⅓ of the ferrous sulfate itself absorbed, were found. In the stool 2 more partially digested tablets were obtained. These tablets had been prescribed for the mother about 4 days previously, and it was estimated that no more than 20 could be missing. At 2:00 a.m. the patient arrived at the hospital showing a gray cyanosis. She was completely limp and unresponsive, and her skin showed many blotchy ecchymoses. Coarse rhonchi, assumed to be due to aspirated vomitus, were audible over the right portion of the chest.

The blood pressure was unobtainable, and the respirations were slow and labored.

*From the Division of Pediatrics and the Department of Pathology, University of Vermont College of Medicine.
†Associate, Mary Fletcher Hospital.
‡Instructor in pathology, University of Vermont College of Medicine.
§Intern, Medical Service, Children's Medical Center, Boston; formerly, intern, Mary Fletcher Hospital.

A chest x-ray film showed slightly increased perihilar markings.

The child received 0.25 cc. of Coramine intravenously on admission, and a transfusion of 250 cc. of plasma was started. Although the respirations were adequately strong and there was no major obstruction of the airway, the cyanosis did not respond to oxygen. At 3:35 a.m. in the belief that she had a methemoglobinemia, 2.8 cc. of a 1 per cent solution of aqueous methylene blue was given, with a definite improvement in color and responsiveness. The blood type was AB, of which none was immediately available. A large specimen for serum iron determination was lost. At 4:30 a.m., while she was receiving 300 cc. of saline solution after the plasma, cyanosis was not entirely gone, and 1.2 cc. of methylene blue was given again in the tubing. At this time she was vomiting and raising fairly large amounts of bloody fluid through her nose. The black, watery stools, continuous since admission, were voluminous and contained oxyuris worms. No response was seen to the second injection of methylene blue, her respirations became less frequent (12 to 14 per minute) and stertorous, although there was little to aspirate, and at 5:10 a.m. she had a slight convulsion and died.

FIGURE 1. *Low-Power View of the Wall of the Ileum, Showing the Iron Pigment Stained Specifically by the Method of Turnbull (Tirmann-Schmelzer Reaction) (Black in the Photograph).*

The iron lies within strips of desquamated surface epithelium (see arrows), overlying and obscuring crests of the villi, and within capillaries and mucosal and submucosal veins. The iron pigment may also be seen in the lumens of the intestinal glands.

At post-mortem examination, performed 5 hours after death, the buccal mucosa and tongue were heavily coated with shaggy-appearing, dirty-brownish-gray material. The peritoneal cavity contained 200 cc. of clear, light-yellow fluid; the linings were smooth, thin and shiny. The pleural cavities were dry. The pericardium contained the usual amount of clear, straw-colored fluid.

The left lung weighed 68 gm. (usual weight, 64 gm.) and the right 98 gm. (usual weight, 66 to 72 gm.) They were crepitant in all lobes, but edematous. Small hemorrhages, averaging 0.8 cm. in diameter, were seen beneath the pleura and were wedge shaped, with the base directed toward the pleura. Though present in the upper and anterior areas, they were most numerous and prominent in the lower lobes and on the posterior surfaces. The mucosa of the larynx, trachea and bronchi to the smallest recognizable branches was covered by a somewhat mucoid, dirty-appearing, brownish-gray, thick, semisolid material.

The liver weighed 380 gm. (usual weight, 331 to 345 gm.). The cut surface had a mosaic appearance of pale, round areas surrounded by a narrow, bright-red zone.

The esophagus, stomach and small and large intestines contained a dirty-brownish-gray, metallic-appearing semisolid material, which was most prominent in the small bowel. The mucosa of the small intestine appeared necrotic, and large areas had sloughed away. When the metallic material was wiped gently away, pin-head-sized, round, regular areas of bright-red discoloration were seen in great numbers.

The unfixed brain weighed 1030 gm. (normal weight, 1010 to 1042 gm.). The surface vessels were very prominent. The subarachnoid fluid was definitely increased although there was no flattening of the convolutions. Several well demarcated, circular, reddish-brown areas 0.3 to less than 0.1 cm. in diameter were demonstrated in the caudate nuclei when the formalin-fixed brain was sectioned.

The heart weighed 50 gm. (normal weight, 48 to 52 gm.).

The spleen weighed 51 gm. (normal weight, 26 to 28 gm.). The follicles were prominent and the cut surface dark red.

The left kidney weighed 34.5 gm. (normal weight, 39 to 43 gm.) and the right 31.0 gm. (normal weight, 39 to 40 gm.). Both had slightly hyperemic surface vessels. The bladder contained no urine.

The adrenal glands were not remarkable.

Microscopical examination of the jejunal and ileal sections showed the surface epithelial layer to be completely eroded away. An occasional desquamated strip was seen, and its cells contained a brown granular pigment. The villous vessels, which were dilated and packed with red cells, contained a similar pigment, granular and usually golden brown though sometimes grayish brown. This pigment was distributed at the vessel periphery, apparently overlying or lying within the endothelial vessels. There was nowhere definite evidence of pigment within the actual cell walls. Irregular, clumped masses of the pigment were also found lying within the lumens of the vessels. The deeper and larger vessels of the mucosa and those of the submucosa were very prominent. The veins were numerous and engorged, containing larger pigment masses, fine granules at the center and coarser granules at the periphery of the masses. Again, some pigment was layered close to the wall and in the same questionable relation to the endothelium as in the villous vessels. There was no pigment in any arterial vessel.

In the lumens of the intestinal glands the pigment was also seen, but never within the cells lining them (Fig. 1 and 2). The lining cells were intact and viable in contradistinction to the sloughed, dying surface epithelial cells.

The mucosa of the ileum, and to a much lesser extent of the jejunum, was lightly and diffusely infiltrated with mononuclear cells, mainly of plasma-cell variety.

One section of ileum showed almost complete sloughing of the mucosa down to the muscularis mucosa, but this was not the usual picture. In another area there was extensive recent hemorrhage into the mucosa, with sloughing of the villi and intact mucosa beneath.

The lymphoid follicles revealed marked reticuloendothelial hyperplasia.

Sections of stomach demonstrated a loss of surface epithelium and a general appearance similar to that described in the small intestine, but to a much less extent. It is significant that the secreting cells of the gastric mucosa showed no pigment ingestion or damage.

The Turnbull blue method of staining for ferrous iron was used.[16] (It stains all reduced iron a deep blue.) This method

demonstrated that all pigment described in the routine hematoxylin and eosin preparations was iron (Fig. 1).

Two phenomena were revealed in the liver sections. Degeneration of the parenchymal cells involved the central four fifths of the lobule, evidenced by poorly staining, light pink, irregularly distributed granular cytoplasm and usually normal nuclei. (There were no fatty changes, and the bile ducts were not remarkable.) The second phenomenon was the presence of irregular masses of granular iron pigment in the lumens of the dilated portal veins (Fig. 3).

The Kupffer cells were large and viable and apparently did not contain iron. No granular iron masses were noted in the central veins.

Sections of kidney revealed small masses of granular material in the capsular space, and larger masses within the lumen of the tubules. The vessels contained none. This pigment stained a light blue with Turnbull's method, possibly because it was Zenker-formalin-fixed tissue, which is not as satisfactory for this method as tissue fixed in 10 per cent formalin.

Immediately surrounding the small terminal bronchioles in the lungs was an area of atelectasis. Peripheral to this

FIGURE 2. *A High-Power View of the Mucosa of the Ileum from the Same Section as That in Figure 1.*

The stained iron pigment is seen within the lumens of the glands. Note its absence within the normal-appearing epithelial cells lining them, as compared with the surface epithelial cells. There is a small vascular channel just off the center of the field, containing several irregular masses of iron pigment of varying sizes. This tendency to line up along the endothelium is characteristic of the iron in most capillaries and vessels studied.

was a zone of edema, congestion and hemorrhage, with obliteration of the normal alveolar architecture. There were only a few inflammatory cells. Some of the bronchioles contained red cells and fluid and were obstructed, infolded and ruptured.

Sections of heart disclosed prominent, increased, dilated and congested intramural capillaries.

Many representative sections of the brain were cut, including several from the caudate nucleus. There were no evident parenchymal changes, and no iron pigment could be demonstrated in the brain. Blood vessels, however, showed very prominent Virchow-Robin spaces, some containing a small amount of light pink, staining, very slightly granular material. All vessels were extremely engorged with blood.

Sections of the bone marrow, spleen and adrenal glands were not remarkable.

Analysis of the intestinal contents and the kidneys gave no evidence of heavy metals. No chemical analysis was done for iron.

Samples of blood taken before death for serum iron and methemoglobin determination were unfortunately lost. An analysis of blood taken after death for methemoglobin was done by Dr. Robertson, of the Biochemistry Department of the University of Vermont College of Medicine, using the method of Evelyn and Malloy.[16] The extinction of the band at 635 millimicrons is measured before and after quenching

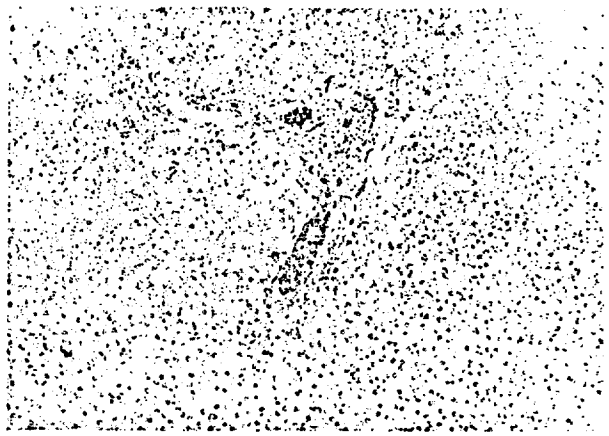

FIGURE 3. *Low-Power View of the Portal Area of the Liver, Showing Masses of Stained Iron Pigment (Black) Lying Within Slightly Dilated Portal Venous Channels.*

Iron could not be demonstrated in the central veins (not shown in the photograph) or in the sinusoids.

with cyanide. In this case the accuracy of the determination was impaired by the presence of methylene blue.

Some consideration was given to the capsule of the ferrous sulfate tablets. The manufacturers of the tablets furnished the information that they are a standard *U.S.P.* preparation and have a gastric-resistant coating. This is a universally used coating and consists of cane sugar with small quantities of starch, calcium, acacia, gelatin and shellac. The color is a certified food dye. All the coating constituents are considered to be therapeutically inert.

DISCUSSION

Certain microscopical features are prominent. In the first place no iron pigment is seen in any vessels (or tissue) beyond the portal veins in the direction of blood flow except in the glomerular spaces and renal tubules. Specifically, none is seen in the central veins of the liver, nor in any of the Kupffer cells lining the sinusoids. The assumption is that the iron at this point goes into solution, presumably combined with serum protein.

In the second place, the assumption that there is a selective affinity of the intestinal mucosal cells for iron is strong. This is evidenced by the taking up of iron by the surface epithelial cells, which have died and have been sloughed off. On the other hand, iron pigment is seen consistently in the lumens of the intestinal glands, but none has been taken up by the glandular epithelium, which is viable and healthy. This selective capacity on the part of the intestinal mucosa is of considerable interest in the

light of the work of Hahn et al.[17] and Granick.[18, 19] The latter has suggested that a protein, apoferritin, is the hypothetical mucosal acceptor of iron of Hahn, producing ferritin. When the mucosal cells had been saturated with ferritin iron, the absorption of iron would be stopped until the body demand decreased the stores, and then absorption of iron would be resumed. Thus is postulated a mechanism of mucosal block or control of ferrous iron. We believe that the evidence presented above indicates the surface epithelial cells and not the glandular cells as the mucosal site of apoferritin and ferritin.

However, regarding the toxicity of ferrous iron, the "mucosal block" is not necessarily and, indeed, probably not effective when large amounts of iron are involved, as in the case reported above. It is suggested that the "barrier" of surface epithelium, with its controlling mechanism, is abolished by the initial nonspecific escharotic effect of sulfate and sulfurous compounds, thus allowing uncontrolled and very rapid absorption of the iron salt.

Gastric irritation is usually considered a characteristic of all oral iron preparations. Ferrous sulfate is no exception. The case reported above, as well as those in the literature, showed severe hemorrhagic gastritis and upper enteritis. Vomiting appeared early and became red, with gross blood. Autopsy showed generalized necrosis of the gastric mucosa. The black, watery, metallic-smelling diarrhea probably indicated irritation farther down as well. Foucar et al.[14] were unable to demonstrate gastrointestinal absorption of iron and therefore concluded that iron per se had no part in the mechanism of toxicity. The Prussian-blue method that they used demonstrated only ferric iron. Had they added Turnbull blue we believe they would have demonstrated the absorbed ferric form.

From the vomiting, diarrhea and enteric hemorrhage there is marked fluid loss. This may explain the shock-like state that the patients present. However, it has been suggested to us that large doses of iron, parenterally at least, can produce a nitritoid reaction. Heffter[20] refers to marked blood-vessel dilatation and capillary paralysis "as with arsenic." Mazur and Shorr[21] postulated a vasodepressor substance in certain types of shock and demonstrated that it was ferritin.

Clinically, the gray cyanosis, unresponsive to oxygen and very responsive to methylene blue, seems best explained as due to methemoglobinemia. Here, again, Heffter's work is corroborative. It states that there can be methemoglobin and hematin formation with both ferric and ferrous salts. We hope that the next observer will not lose his antemortem blood sample. The dual role[22] of methylene blue, reverting methemoglobin in small concentrations and producing it if the dose is greater, may explain the failure of the second dose in this case.

Questions about the effect of iron intoxication on the blood-clotting mechanism are raised by the scattered hemorrhages usually found at autopsy and the fact, in the case reported above, that the heart's blood was unclotted five hours after death.

Russell[12] pointed out evidence of renal impairment, and cloudy swelling of renal cells was described in this case.

Beyond general supportive measures and transfusion, the problem of proper therapy in ferrous sulfate toxicity is a difficult one.

BAL was not used because Randall and Seeler,[23] although not mentioning the ferrous ion, stated that the toxicity of certain metals is enhanced by combination with BAL. Work by Edge and Somers[24] on mice indicated that the iron combination has this effect, and its use by Thomson[25] was not beneficial. Somers[26] believes that Thomson's use of sodium bicarbonate lavage would be useful only immediately after ingestion and prefers a demulcent by mouth, aluminum hydroxide combined with bismuth sulfate and a central emetic.

The death described seems definitely to have been due to ferrous sulfate poisoning, and not to any capsular or other foreign ingredients. The strict directive that the pill bottle be kept out of reach of children should go with the dispensing of this, as well as with most pharmaceuticals. Perhaps the popular magazines should make the warning more widespread that even ferrous sulfate can be lethal.

The best management of these cases needs clarification. Intubation, gastric lavage and gastric precipitation of remaining drug is difficult in the face of severe vomiting, and perhaps is dangerous with the hemorrhage and necrosis present in the stomach. BAL is apparently not acceptable.

Whole blood is definitely indicated in the correction of this type of shock, and perhaps epinephrine would be the best stimulant.

If methemoglobinemia proves to be a regular part of the picture, Finch[27] recommends methylene blue as the perfect antidote, a fraction of a milligram to 10 mg. per kilogram of body weight. His advice was followed in the treatment of the case reported above. Whole-blood transfusions would, of course, help.

First aid in the home would probably be prompt emesis and feeding of raw eggs or milk, so that protein could absorb the iron. Hospitalization should be prompt, and there apomorphine would be available for early cases.

Summary

A fatal case of ferrous sulfate toxicity in a seventeen-month-old child, with autopsy findings, is presented. The patient exhibited signs of severe enteric irritation, vasomotor collapse, gray cyanosis unresponsive to oxygen but rapidly responsive to methylene-blue therapy, scattered hemorrhages and possible renal and pancreatic damage.

Iron absorption was shown by Turnbull-blue technic.

A hope is expressed that this report will stimulate the report of similar cases, and it is suggested that the toxicity of this drug, generally considered innocuous, be better publicized.

We are indebted to Dr. Rosemary Brewster for her assistance on this paper.

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THE PATHOLOGY OF FERROUS SULPHATE POISONING

J. P. SMITH

From the Department of Pathology, University of Manchester

(PLATES XCVIII AND XCIX)

THE increased therapeutic use of iron during the past few decades has brought the problem of poisoning with iron preparations into prominence.

The toxicity of iron salts has long been recognised; a case of ferrous sulphate poisoning in an adult was described by Limouzin-Lamothe (1850) and two further cases by Chovallier (1850; 1858). Other adult cases of ferrous sulphate poisoning have been described by Foucar, Gordon and Kaye (1948), who also quote cases described by Hall in 1883 and by Fitts in 1888-89. Meyer and Williams (1831) first demonstrated the toxic effects of intravenous administration of various iron salts into animals. Starkenstein (1926) determined the ratio of the toxicity by parenteral administration to that of oral administration; for the ferrous salts it was 1 in 5 to 1 in 10 and for the complex iron salts, 1 in 100.

The more recent cases of ferrous sulphate poisoning have occurred in young children for whom the temptation of a green, sugar-coated tablet is considerable. Forbes (1947) first described 2 cases in infants and a further 15 cases have since been reported (Roxburgh, 1949, 1 case; Thomson, 1950, 6 cases; Spencer, 1951, 8 cases). Of these 17 cases 8 have proved fatal. This mortality rate is misleading, since it seems certain that many non-fatal cases have not been published (Lodge, personal communication, 2 cases; Woodcock, personal communication, 2 cases).

In most of the previously published cases the pathological study has been brief and incomplete. In this paper I record a further fatal case in which a more detailed pathological study has been made and offer a tentative explanation of the mechanism of ferrous sulphate poisoning.

CASE REPORT

A male child aged 21 months was discovered about 9.30 a.m. to be playing with a box of ferrous sulphate tablets which he had got from an elder brother about an hour previously. Of the 84 tablets which the box had contained 41 could not be accounted for and were presumed to have been swallowed by the child. He was admitted to the Manchester Royal Infirmary one hour later in a state of profound shock. The skin and mucous membranes were cyanosed, the pulse was rapid and regular and the respirations were bubbly, with coarse

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rales in the lower chest. The child was restless and seemed to have some abdominal pain. The stomach was washed out with sodium bicarbonate; the washings were dark brown and were later shown to contain large quantities of iron. The patient's condition rapidly deteriorated; he lapsed into coma, death occurring 4 hours after the ingestion of the tablets.

Autopsy findings

The body, examined 21 hours after death, was that of a well-nourished male infant of average build. There was intense cyanosis of the nail-beds and lips but no jaundice or other external evidence of disease or injury.

Stomach. Dilated, contained 16 ml. of greenish-black mucoid fluid streaked with blood. The small intestine contained 100 ml. of thin, greenish-black fluid, the large intestine 50 ml. Contents gave strongly positive tests for iron with acid ferrocyanide and left much iron residue when ashed. No tablets, or portions of tablets, could be recognised in them.

The whole of the mucous membrane of the stomach was congested, red-brown in colour and covered by a thick layer of mucus. The superficial layer of the mucosa was necrotic and petechial hæmorrhages were present throughout. The peritoneal surface showed many dilated and congested vessels.

Intestines. Mucosa of small intestine congested, especially in the first and second parts of the duodenum, where the crests of the rugæ were brown and necrotic. Peyer's patches looked normal; the large bowel showed no abnormality.

Other organs. Liver (420 g.) pink, normal in consistency and pattern; 20 ml. of serous fluid in the pericardial sac; right auricle and ventricle dilated, with flabby myocardium; the left ventricle firm and contracted. No valvular defects. Spleen and kidneys congested. The remaining abdominal viscera—pancreas, adrenals, ureters and gall-bladder—were normal.

The bladder was contracted and empty. Pleural cavities contained no free fluid; no abnormality in the lungs, pharynx, larynx, trachea, oesophagus, thyroid gland or thymus. The brain, meninges and hypophysis appeared healthy and the lymph glands throughout the body were normal. Active red marrow was present in the femoral shaft.

Histological appearances

The gastric mucosa is congested and shows patchy, superficial necrosis with petechial hæmorrhages and occasional polymorphonuclear leucocytes amongst the tubular glands. Perls's reaction for iron shows an intense impregnation of the reticulum of the superficial third of the mucosa, of the basement membrane of the capillaries, lymphatics and venules and of the endothelial cells of the venules

FIG. 1.

FIG. 2.

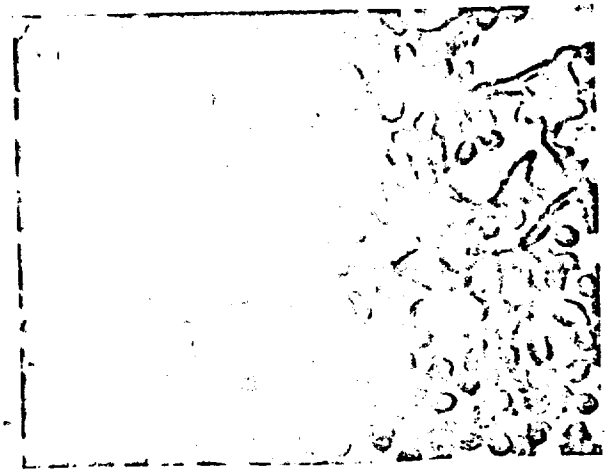

FIG. 3.

PLATE XCVIII

FIG. 1.—Section of gastric mucosa. The reticulum of the superficial third of the mucosa and the endothelial cells of capillaries and veins show intense iron impregnation. Perls's reaction. ×40.

FIG. 2.—Veins in submucosa of stomach showing iron in the endothelial cells and granular platelet thrombosis encroaching on the lumen. Many of the perivascular collagen fibres are also impregnated with iron. Perls's reaction. ×260.

FIG. 3.—Littoral cells of a gastric lymph follicle containing granules of iron. Perls's reaction. ×800.

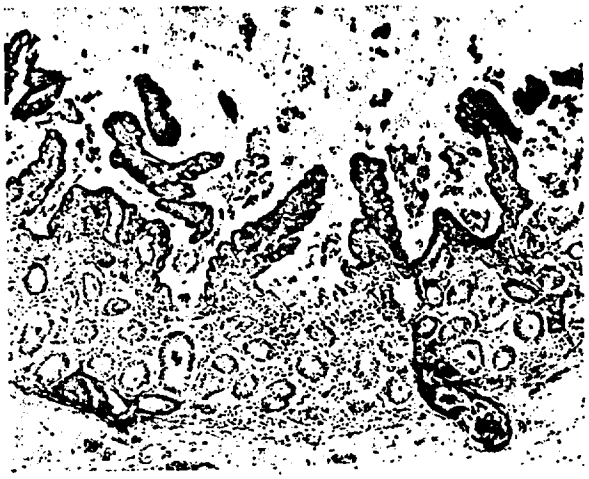

FIG. 4.

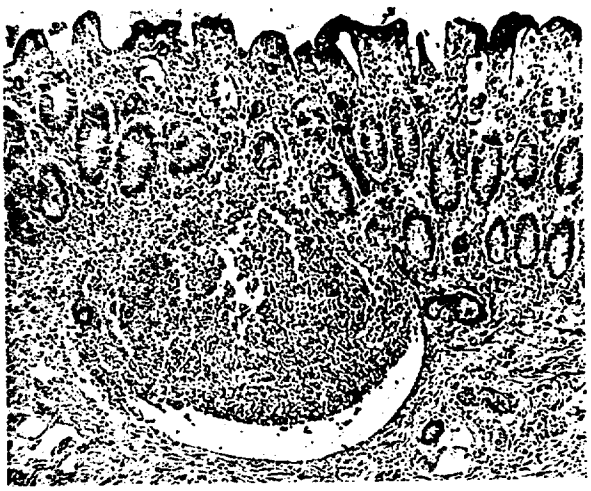

FIG. 5.

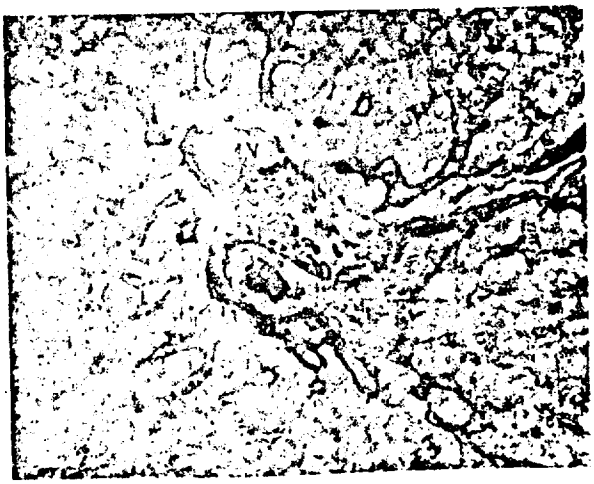

FIG. 6.

PLATE XCIX

FIG. 4.—Section of jejunum showing necrosis of the mucosa with iron impregnation of the reticulum and of the venous endothelium. Perls's reaction. ×65.

FIG. 5.—Iron impregnation of the mucosa of the colon. Iron is also present in the endothelium of the veins but not in the lymph follicle. Perls's reaction. ×70.

FIG. 6.—Section of a portal tract showing iron in the portal venous endothelium and in the reticulum of the hepatic sinusoids. An iron-impregnated embolus lies in the portal vein. Perls's reaction. ×310.

(fig. 1). There is slight œdema of the submucosa, with scanty polymorphonuclear leucocytic infiltration, and many of the collagen fibres around the veins are impregnated with iron. The most striking feature is the heavy deposition of granular iron in the endothelial cells of the veins of all coats, including the large vessels of the subserosa. Deposited upon the damaged endothelium is a granular amorphous material varying in amount from an annular rim (fig. 2) to a mass completely occluding the lumen. This material, which is composed of uniform, granular particles the size of platelets, does not give the usual staining reactions of fibrin and appears to be platelet thrombus; it frequently contains masses of particulate iron. Small lymph glands in the subserosa show granules of iron in the littoral cells of the sinuses (fig. 3). The muscle fibres are not affected.

Focal superficial necroses and petechial hæmorrhages are present in the mucosa of the duodenum, but that of the rest of the intestines is normal, apart from iron impregnation of the superficial layer throughout (figs. 4 and 5). The submucosal venous endothelium in the jejunum and colon, but not in the ileum, contains iron particles. Platelet thrombosis in varying degree has occurred in these veins. Brunner's glands in the duodenum and the lymphoid follicles in the intestine contain no stainable iron.

The hepatic parenchyma is normal and contains no demonstrable fat or iron. An excess of polymorphonuclear leucocytes is present in the sinuses. The endothelial cells of many portal veins contain granular iron and there is irregular iron impregnation of the reticulum of the periportal hepatic sinuses. Loose embolic masses of platelet thrombus containing particulate iron are present in some branches of the portal veins (fig. 6).

The pulmonary capillaries are congested, as are those of the kidneys, adrenals, spleen, pancreas and hypophysis. In the lateral wall of the third ventricle, above the corpora mammillaria, three small arterioles are heavily cuffed with lymphocytes. The nature of this lesion is obscure but it appears to have no relationship to the primary condition. Many further sections of the brain, the myocardium, thyroid, thymus, bladder, œsophagus and bone marrow show no abnormality and stainable iron cannot be demonstrated in any of these organs.

Discussion

The autopsy findings in this case agree substantially with those of previous reports but these do not mention the iron impregnation of the portal venous endothelium and periportal hepatic reticulum, or the presence of iron-laden platelet emboli in the portal veins. The iron staining of the reticulum of the gastro-intestinal tract seems to be due to direct diffusion of iron into the damaged mucosa. The presence of iron in the submucosal venous endothelium and the iron

impregnation of the platelet thrombi indicate a heavy intravenous concentration, which explains the appearance of iron in the liver. Support for this suggestion is obtained from Spencer's (1951) finding in two of his cases, of serum-iron levels of 3·3 mg. per cent. and 3·4 mg. per cent. (normal 0·035-0·22 mg. per cent.) 4 and 5 hours after ingestion of the tablets. Platelet emboli in the liver are not sufficiently numerous to have seriously affected the organ and no emboli are present in the pulmonary or systemic circulations. Cloudy swelling, fatty changes and necrosis of the liver described in 4 of the 5 previous cases in which histology is recorded, are completely absent in the present case. By direct diffusion, iron appears to have entered the lymphatics of the gastric mucosa, whence it has been transported to the regional glands and deposited in the littoral cells of the sinuses.

To study the method of absorption and transport of this iron, sections were also stained with acid ferricyanide (Turnbull's method for ferrous iron). Sections of all parts of the gastro-intestinal tract showed the reaction to be most marked toward the surface of the mucosa, the distribution being similar to, though less extensive than, that in corresponding sections stained for ferric iron. In the liver, only faint staining of the reticulum could be obtained by this method. Granick (1946*a*) has shown that the oxygen tension in the tissues is sufficient to oxidise any free ferrous ions to the ferric state. The above results, therefore, suggest post-mortem reduction of ferric iron rather than in-vivo impregnation of ferrous iron.

The preparation responsible for the reported deaths contains ferrous sulphate 3 grains, manganese sulphate 1/25 grain, and copper sulphate 1/25 grain in each tablet. Forbes (1947) has proved that ferrous sulphate is the sole toxic agent in this preparation.

Neither the gross nor the histological changes in the present case or in the previously reported cases offer a satisfactory explanation of death. Forbes (1947) and Prain (1949), on the basis of cloudy swelling, fatty changes and necrosis in the liver, concluded that death was due to the toxic effect on the hepatic parenchyma of substances absorbed from the damaged gastric mucous membrane. Somers (1947), in experimental work on guinea-pigs and rabbits, found no constant hepatic damage and concluded that the histological changes were insufficient to account for the death of his animals.

A clinical study by Spencer of the reported cases has shown two critical periods in the illness when death is apt to occur. Four of the deaths occurred within 6 hours of taking the tablets, and 5 between 20 and 53 hours. The rapidity of death in the first group suggests the presence of some highly potent active agent for which the gastro-intestinal damage alone seems hardly adequate. Most of the early clinical signs—pallor, cyanosis, coldness, tachycardia and restlessness—are those of peripheral circulatory failure.

Recent work on the role of ferritin in the absorption of iron and on the identification of ferritin with the vaso-depressor material (V.D.M.)

of the shock syndrome suggests a new explanation of the mechanism of ferrous sulphate poisoning.

The absorption of iron from the intestinal tract is now known to be regulated according to the iron requirements of the body. The mechanism has been explained by Hahn *et al.* (1943) and Granick (1946*b*), who have demonstrated, by means of radioactive iron and histochemical methods respectively, a " mucosal block ", the essential factor in which is the substance " ferritin ". Ferritin consists of micelles of ferric hydroxide attached to a soluble specific protein, " apoferritin ". This protein cannot be demonstrated in normal intestinal mucosa and only appears in response to the absorption of iron into the mucosal cells. Combination of iron and protein immediately occurs and absorption continues until there is physiological saturation of the mucosal cells with ferritin, after which further absorption of iron is prevented (Granick, 1946*c*). Ferritin cannot be demonstrated in the blood of normal animals (Granick, 1943) and the mode of transfer of iron to the blood and body tissues is not adequately understood. Granick has suggested that iron is released from ferritin directly into the blood, where it combines with globulin. In the liver, spleen and bone marrow, he has shown experimentally that iron is in some way reconverted into ferritin and stored.

Failure of the blocking mechanism under certain abnormal conditions has been demonstrated by many workers, and recently Hegsted *et al.* (1949) and Kinney *et al.* (1949) have shown that the absorption of iron is also related both to the absolute amounts of iron and phosphate in the food and to the ratio of iron to phosphate. Either an excess of iron or an insufficiency of phosphate will increase the amount of iron absorbed.

In addition to its role in the control of iron absorption and storage, ferritin has been shown to have a marked vaso-depressor activity. Shorr, Zweifach and Furchgott (1945) have demonstrated an active vaso-depressor material (V.D.M.) in the blood of animals in the experimental shock syndrome and have postulated that this material is an essential factor in the human syndrome. Mazur and Shorr (1948), using a combination of chemical and immunochemical procedures, together with the rat mesoappendix test of Zweifach (1948), identified this vaso-depressor material as ferritin.

It now seems reasonable to postulate that massive ingestion of iron, as in these children, will overpower the normal mucosal barrier and an excess of iron will enter the mucosal cells. Excessive production of ferritin in these cells may occur, and some may escape into the circulation. Much of the excessive serum iron may similarly be converted into ferritin in the liver, spleen and bone marrow, and excessive production here may also release it into the circulation. In this way, the shock, which is an important feature of the first phase of ferrous sulphate poisoning, would be both initiated and maintained.

The inhibition of iron absorption by phosphate suggests the possible value of a phosphate salt in the treatment of cases of ferrous sulphate poisoning.

Summary

1. The pathological findings in a case of ferrous sulphate poisoning in an infant are described.

2. The role of ferritin in the absorption of iron and in the genesis of the shock syndrome is briefly discussed.

3. It is postulated that in acute cases of ferrous sulphate poisoning there may be an overproduction of ferritin in the body. This will initiate the shock syndrome from which death finally occurs.

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RELATIVE ORAL TOXICITY OF SOME THERAPEUTIC IRON PREPARATIONS

BY

G. F. SOMERS, B.Sc., Ph.C.

Research Division, Glaxo Laboratories Ltd., Greenford, Middlesex

Forbes (1947) and Thomson (1947) have recently described serious consequences in young children, including three deaths, following the unauthorized taking of ferrous sulphate tablets in excessive numbers. These and certain cases previously reported to us raised the general question of the toxicity of iron compounds when large doses were consumed orally, and also of the additional effect, if any, of the small amount of copper and manganese sulphates which were present in the tablets.

Examination of the literature failed to reveal earlier reports of ill effects from orally administered iron compounds, except for occasional references to slight alimentary disturbances. Further, and more surprisingly in view of the widespread use of iron compounds for the treatment of iron deficiency or "nutritional" anaemia, we have been unable to find any account of pharmacological investigations into the action of iron given by mouth, although its effects by injection have received some attention and it is accepted that serious ill effects may follow its absorption into the blood stream.

It therefore seemed desirable to test experimentally on several species of laboratory animal the effects of orally administering very large doses of some of the more commonly used iron preparations. For this purpose we chose ferrous sulphate, with and without the presence of the "trace" metals copper and manganese; ferrous carbonate in the form of Blaud's pills; ferrous gluconate, because of the claims made for it by Reznikoff and Goebel (1937); ferric chloride, so as to include at least one preparation of dializable ferric iron; and iron and ammonium citrate, widely used in compounded medicines, though nowadays largely replaced by ferrous sulphate for simple oral iron therapy—shown by many workers from Davidson (1933) onwards to be much more effective in haemopoiesis than the non-ionized "scale salts," when compared on an equivalent iron basis. Rabbits and guinea-pigs were used for studying the pathology and histology of any disturbances observed, and mice for the estimation of median lethal doses (LD50) with reasonable accuracy.

As a practical problem in human therapeutics we were really concerned with establishing the "therapeutic index" of ionizable iron—that is, the ratio of its median toxic dose to its median therapeutic dose. Here we encountered two difficulties, the one general and the other particular. First, it is ordinarily impossible to establish such an index for the action of a drug on the human organism, simply because data, unfortunately, are not as a rule available for the median toxic human dose. The pharmacologist therefore normally has recourse to establishing the therapeutic index of a drug on at least one species of laboratory animal, and preferably on more than one. If the value of this index is of roughly the same order for two or more species of laboratory animals it is reasonable to assume that the same value will apply to man; in general, experience bears out this assumption. However, even this line of approach was not available to us, for there is no species of animal—with the possible exception of the rat made anaemic on a milk diet—for which we have information about the median therapeutic dose of iron compounds.

We were consequently forced back on to the unsatisfactory step of relating toxic doses to the body weight of the animal used and arguing on the same basis for man. The objections to this line of reasoning, however, appear much less strong for iron compounds than they might for other drugs, for it will be seen that toxic effects—and, in particular, fatal results—were produced only by doses so immensely out of proportion to the animal's normal intake of iron as to make highly unlikely the ingestion of dangerous amounts by human subjects. The facts established by our experiments nevertheless make it clearly desirable to render access of infants and small children to iron preparations as difficult as possible. Such precautions would normally be taken with many of the common drugs to be found in the ordinary household—for example, quinine salts and acetylsalicylic acid—without in any way detracting from their value and importance in therapeutics.

Toxicity

The results obtained with the three species of animals used are summarized in the accompanying table. The

Table showing the Median Lethal Toxic Doses of Iron Compounds by the Oral Route

Compound	Iron in Preparation, Calculated as Fe	Dose per kg. of Body Weight					
		Rabbits		Guinea-pigs		Mice	
		Actual	Cal. as Fe	Actual	Cal. as Fe	Actual	Cal. as Fe
	%	g.	g.	g.	g.	g.	g.
Ferrous sulphate crystals	20	3·0	0·6	1·5	0·3	4·5	0·9
Ferrous sulphate prep. with copper and manganese	24	3·0	0·72	1·25	0·3	4·1	1·0
Ferrous gluconate ...	16·6	3·5	0·58	2·1	0·35	6·6	1·1
Ferrous carbonate pills	12·5	17·8	2·22	16·0	2·0	31·0	3·8
Ferric chloride ..	34	1·2	0·4	0·6	0·2	1·5	0·5
Ferric and ammonium citrate	20	2·8	0·56	1·75	0·35	5·0	1·0

figures from tests on rabbits and guinea-pigs, owing to the relatively small numbers of such animals that can be used in toxicity tests, must be regarded as first approximations; the figures from the tests on mice have a greater accuracy, except those for iron and ammonium citrate, because with this product a great individual variation was found among the animals. Slopes of the regression curves, and the errors of estimating the LD50, which were of the order usually found in tests of this kind, were obtained by the graphical method of de Beer (1945).

It will be seen: (1) That the presence of small amounts of copper and manganese sulphates made no difference to

the toxicity of the iron of ferrous sulphate. (2) That the iron in ferrous sulphate, ferrous gluconate, and—curiously enough—iron and ammonium citrate had the same toxicity, though it must be repeated that the figure for the last compound is subject to a very large error. (3) That the iron in Blaud's pills has apparently only about one-quarter the toxicity of the other forms. It is, of course, well known that the iron in Blaud's pills has also much less therapeutic effect, and these two facts are undoubtedly due, at least in part, to the same basic phenomenon—the relative insolubility of their ferrous carbonate in gastric and intestinal contents. (4) That ferric iron appears to be up to twice as toxic as ferrous iron. We have no explanation for this finding, especially if, as is generally held, ferric iron is reduced in the stomach to the ferrous state. It must, however, be remembered that ferric chloride is a much more acidic substance than ferrous sulphate (or gluconate). The introduction of large quantities into the stomach must involve a considerable increase in gastric acidity, which in turn may hasten alimentary absorption of the iron—possibly an advantage with therapeutic doses but certainly not with toxic ones.

The observation with Blaud's pills suggested that sodium carbonate or bicarbonate might be useful as an antidote to toxic oral doses of iron compounds. Four rabbits were therefore given known toxic doses of ferrous sulphate (3 g. per kg. of body weight) and two of them then received the same amount of sodium carbonate. One of these two lived for two days, while the other survived completely; the two that did not receive sodium carbonate died overnight. This experiment was repeated twice, with similar results—a fact which points to the conclusion that sodium carbonate treatment might, if given soon enough, help to reduce the toxic effects of excessive iron doses by mouth.

Pathology and Histology

Rabbits

The rabbits used were of both sexes and weighed between 2 and 3 kg. They were fasted overnight and were then given by stomach tube doses of the iron compounds, graded and proportionate to their body weights. Two animals were used for each dose and at least ten animals for each substance. If the rabbits survived, daily samples of urine were collected for three days. Haematological examinations included differential and total counts of red and white cells and estimates of haemoglobin levels. Necropsies with examination of all major organs followed as quickly as possible after death. Sections were cut of parts of the stomach, intestine, liver, and kidneys, and were stained for iron as well as with haematoxylin and eosin.

Within a few minutes of receiving a toxic dose of iron the rabbits became prostrated. They lay on their stomachs with limbs extended, their respiratory rate increased, micturition often occurred, and reflex movement of the hind legs was considerably retarded. Coma followed, with shallow breathing and gradual disappearance of reflex movements. The animal either died two to six hours after receiving the dose (according to its size), sometimes following convulsions, or recovered.

The rabbits receiving Blaud's pills had a profuse diarrhoea. This fact, with the freedom of all other animals from this symptom, has, we believe, a very simple explanation. The pills are made by reacting ferrous sulphate with sodium carbonate, so that the ferrous carbonate formed must be accompanied by an equivalent amount of sodium sulphate. There seems no reason why a large dose of Glauber's salts should have an effect on rabbits differing from that on man. Moreover, the laxative effect of the associated sodium sulphate must have hastened the alimentary passage of these large doses of ferrous carbonate; this would tend further to reduce its toxic effects.

At necropsy the stomachs usually showed congested areas with shedding of mucosa, especially at the greater curvature. The amount of damage was to a large extent determined by the size of dose: the smaller toxic doses caused only a slight damage to the stomach wall. Bleeding into the stomach was the exception rather than the rule, but it was seen after the larger doses of both ferrous sulphate and ferric chloride and in one animal that had received ferric gluconate. The small intestines generally showed no hyperaemia in their upper regions, but here also haemorrhages were seen only after very large doses. Surviving animals showed no evidence of kidney damage, the urine being invariably devoid of abnormal constituents. There were no departures from normal blood counts. Recovery of surviving animals was usually rapid.

Changes in the histology of stomach, liver, and kidney in rabbits killed by the iron compounds were very small and appeared insufficient to account for the death of the animals. In the stomach there was only slight necrosis of the superficial layer of the villi and deposits of iron on the mucous membrane (occasionally also in the endothelium of the smaller blood vessels). There were deposits of iron in the bile ducts, but only slight hydropic changes in the liver. In rabbits surviving and killed three days after dosing we observed small foci of fatty degeneration, and necrosis was present in the peripheral parts of the lobuli, with deposits of iron, mainly in the Küpffer cells. The other surviving animals appeared to be completely restored to normal health and activity after a few days.

Guinea-pigs

The animals used, of either sex, weighed between 250 and 300 g. Their distribution over the different dosage groups was made so far as possible to equalize the average weights of the groups. Each dose was given to at least three animals, and at least ten guinea-pigs were used for each substance tested. The animals were fasted overnight and given the appropriate dose of iron compound by dropping it into the mouth and then tickling the fauces. Necropsies, dissections, and histological work were carried out as on the rabbits.

In general the immediate consequences of excessive iron administration by mouth were the same in guinea-pigs as in rabbits. At necropsy, however, there was evidence of severer damage to the stomach; macroscopically the findings with the larger doses resembled those described by Forbes. After doses of 1.5 g. per kg. of body weight of ferrous sulphate or ferric chloride the stomach contained both fresh and changed blood; necrosis, shedding of mucosa, and areas of haemorrhage were obvious to the naked eye. Severe irritation of the stomach wall followed the ingestion of 3 g. of ferrous gluconate per kg. of body weight: in one animal the stomach was full of blood-stained material. Changes were less severe after dosing with iron and ammonium citrate or Blaud's pills, but with the larger dose levels either preparation caused well-marked irritation of gastric mucosa with occasional petechial haemorrhages.

Histological changes, as in rabbits, were slight and by no sufficient in themselves to account for the deaths. There was but superficial damage to the gastric villi, with deposits of iron on the mucous membrane, which also showed scattered areas of capillary bleeding. In a very few instances there were some fatty changes in the liver.

Necropsies were not made on mice, which were used solely to obtain a reasonably accurate estimate of the median lethal doses.

Discussion

From the experiments described there can be no doubt that in very large doses certain soluble iron salts, whether ferrous or ferric, whether of organic or inorganic acids, and whether normally ionizable or complexes of the "scale salt" type, are toxic to at least three species of laboratory animals. It is reasonable, and probably a wise precaution, by extrapolation to accept as proved that all similar iron compounds can, in excessively large doses, kill mammals of any species, including man. It must, however, be emphasized that toxic doses really are excessive. The amount of ferrous sulphate necessary to kill on the average one out of two 10-stone (63.5-kg.) men, if man's susceptibility on a body-weight basis is assumed to be the same as that of the rabbit, would represent at least several hundred tablets of 3 gr. (0.2 g.) of exsiccated ferrous sulphate, each containing 1 gr. (65 mg.) of iron. Obviously the number may be considerably smaller for infants and young children, being reckoned in tens rather than hundreds.

The similarity in behaviour of various iron preparations suggests that after solution in the stomach they are all reduced to the ferrous state and that ferrous iron when present in very large amounts, whether wholly ionized or not, is alone responsible for the damage. Ferrous sulphate should be completely ionized at high concentrations, and ferrous gluconate probably hardly ionized at all even at low concentrations, yet they show indistinguishable toxicities in very high doses. It may be remarked, in passing, that none of the ionizable iron compounds exists as such in the stomach after therapeutic doses: apart from un-ionized hydrochloric acid in the gastric contents, there will then be present in solution the chloride, sulphate, and other anions, the hydrogen, iron, and other cations, and the various un-ionized soluble constituents. Forbes's contrasting of ferrous sulphate with ferrous chloride in solution therefore seems to be misleading even when the sulphate has been ingested at much above the therapeutic level.

The post-mortem and histological examinations have furnished no positive information about the *modus operandi* of iron at orally toxic levels. A decision between shock due to tissue damage—which at worst was not very great and appeared rapidly reversible in the milder cases—and systemic effects following passage of excess iron into the blood stream cannot be made on the basis of our experiments. They were, in any event, carried out with an immediately practical object—to find and record at what doses therapeutic iron preparations could exert toxic effects on experimental animals. The results of these experiments are reassuring. They show, at any rate in so far as the experimental animals react similarly to man, that the gap between curative and harmful doses of iron compounds is very large—and obviously still larger between preventive and fatal doses. Indeed, I doubt if there are many medicinal substances with so large a "therapeutic index." The upper dose of ferrous sulphate, according to the *British Pharmacopoeia*, is 0.3 g., which would be contained in five tablets of the product causing fatalities that led to this investigation. In few circumstances is it likely that an adult would be recommended to take more than 12 such tablets a day, and the results suggest the harmlessness of anything less than several hundred tablets taken all at one time and on an empty stomach. It is, however, desirable that physicians and pharmacists should warn parents and adults generally that iron preparations should be kept out of reach of the very young.

In the experiments described above I have received the technical assistance of Mr. G. A. Romer in the preparation of sections and slides, and much useful advice from Dr. J. Ungar, to both of whom I wish to extend my thanks.

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British Medical Journal

FERROUS SULPHATE POISONING IN CHILDREN

BY

I. O. B. SPENCER, M.B., M.R.C.P.

(From the Children's Department, Royal Victoria Infirmary, Newcastle-upon-Tyne)

Iron poisoning in children occurs through the temptation of attractive sugar-coated pills containing ferrous sulphate which, with good reason, are freely supplied to their mothers. The first comprehensive reports of this disaster in children were published in 1947, and, though it is difficult to believe that cases did not occur before, the first recorded case was that of a child of 16 months who died, in May, 1944, after swallowing 39 ferrous sulphate tablets (Thomson, 1947). Since this time there have been further case reports, and the clinical picture of the poisoning is becoming clear.

The daily press has recently given prominence to inquests on children who have died after swallowing ferrous sulphate tablets, and I have used this source of information to collect some details of four cases to add to the reports of four which I have seen myself. Brief reference has already been made to two of these coroners' reports (Cases 6 and 8) in a leading article in the *Lancet* (1949). The daily press has also made reference to the ignorance of parents and doctors alike concerning the effects and dangers of these tablets to small children.

It is the purpose of this paper to describe the clinical picture of iron poisoning in detail, to discuss the effects of large doses of iron, to propose a more positive therapeutic approach than has been given in previous medical articles, and to suggest a method of safeguarding children from these tablets.

The Tablets.—The most readily available tablets are green sugar-coated pills, and each of these contains ferrous sulphate, 3 gr. (0.2 g.), copper sulphate, 1/25 gr. (2.6 mg.), and manganese sulphate, 1/25 gr. (2.6 mg.). Forbes (1947) has shown by animal experiments that the effect of the copper and the manganese can be discounted, and that it is the ferrous sulphate which has the irritating and lethal effect.

The cases recorded below have, with the exception of the coroners' cases, all been treated is this department within the space of a year. In this period three other children were admitted with a diagnosis of ferrous sulphate poisoning, but they have been excluded because of lack of detail regarding what they had swallowed. One had the symptoms of poisoning, but the other two were not upset. The mother of one of the latter, aged 18 months, was quite sure he had swallowed 24 tablets, but only minute quantities of iron were recovered from the stools and stomach washings, and it was thought more probable that only a few pills had been taken than that 24 tablets had not made him ill.

In the seven preceding years during which this department has been in existence only one other such case has been admitted. This was a girl aged 2½ years who swallowed 60 tablets. Her mother thought she vomited back "nearly all the tablets," and, though she was pale and drowsy and had marked diarrhoea and vomiting, her recovery during the subsequent three days seems to

have aroused no special interest at the time. Her case has been excluded owing to the doubt about the number of tablets she retained.

Case 1

Between 5.30 and 6.10 p.m. on June 26, 1950, a girl aged 21 months swallowed 75 ferrous sulphate tablets. These made her sick, and her father, realizing what had happened, held her upside down over the kitchen sink, whereupon she vomited 21 tablets. After this a nurse gave her some salt and water to drink, and she was sick again. Later the family doctor washed her stomach out with normal saline, but on neither occasion were further tablets returned. From 6.15 p.m. the child was said to have been cold, pale, and semi-conscious.

At 8.35 p.m. she arrived in hospital, and her condition was that of a well-nourished but shocked child. She was cold and pale, with slightly cyanosed lips, and her pulse was rapid (150 a minute) and difficult to feel. She was semi-comatose in that she showed little resentment to painful stimuli, but she did open her eyes momentarily when roused. Further examination revealed no abnormality. Gastric lavage was carried out with normal saline, and 8 oz. (230 ml.) of sodium bicarbonate solution was left in the stomach. During this manipulation the returning fluid contained shreds of tissue and streaks of blood but no further tablets.

For the next 10 hours the child was unaware of her surroundings. She was very restless and frequently retched and vomited small quantities of dark red blood. Her respirations were irregular, shallow, and rapid (60–70 a minute) and her temperature rose to 102.8° F. (39.3° C.).

By 6.30 a.m. on June 27 she seemed a little better and was warm, though still pale and very restless. She was retching frequently but not vomiting quite so often, and for the remainder of the day her condition remained much the same. She occasionally showed a little interest in her surroundings and appeared to recognize her father. By evening she was drowsy and slept for short spells, but was very restless in the intervals. She was vomiting "coffee-ground" material but also taking drinks of water. Her bowels were opened three times, the stools being small, dark, and very offensive.

On June 28 she was taking a little more interest in her surroundings and tried to sit and stand up occasionally, but she was very "floppy" and seemed too weak to do so. She vomited "coffee-ground" material on two occasions, but was very drowsy and slept most of the time. Next day the improvement was maintained. She slept well through the night and was still drowsy during the day. No further vomiting occurred. On the 30th she was much more lively but still rather "floppy," and could not sit up on her own yet.

On July 3 her behaviour was normal, and she was discharged from hospital on July 7. A fortnight later her appetite was poor, and she had vomited at least once a day at home. Thereafter vomiting ceased, and on August 31 the mother said that her child "has really been back to her normal self for the past week."

Investigations.—June 26: 10.30 p.m., serum iron, 3.3 mg. per 100 ml. (normal, 0.035–0.22 mg. per 100 ml.). *June 27:* 1.30 a.m., serum iron, 2.25 mg. per 100 ml.; 9.30 a.m., serum iron, 1.12 mg. per 100 ml.; 10.15 a.m., B.P., 120/70; haemoglobin, 68%; 10.30 a.m., E.C.G., normal; 5 p.m., E.C.G., normal; 10.30 p.m., B.P., 105/60. *June 28:* 10 a.m., B.P., 135/70; haemoglobin, 60%; urinary iron content, *nil*; 3 p.m., urinary iron content, *nil*. *June 29:* 10 a.m., B.P., 120/65; haemoglobin, 62%; E.C.G. normal; 12.30 p.m., serum iron, 0.2[illegible] mg. per 100 ml.; urinary iron, 0.45 mg. per 24 hours. The urine contained a trace of albumin; microscopy showed nothing abnormal. *August 31:*—Liver-function tests: zinc sulphate, 9 units; thymol turbidity, 12 units; thymol flocculation, +++; cephalin-cholesterol, +++; alkaline phosphatase, 23.8 units.

Case 2

At 11 a.m. on February 2, 1951, a boy aged 23 months was found to have eaten 16 ferrous sulphate tablets and 10 iron plastules (the total iron content of tablets and plastules was 98 gr. (6.5 g.) of ferrous sulphate, or the equivalent of 33 ferrous sulphate tablets). He was immediately given salt and water to drink, and he vomited some "black liquid slime" containing some partly dissolved plastules and four tablets. In the next hour he vomited several times and then went to sleep. When he awoke at 1.45 p.m. he vomited about a tablespoonful of bright red blood, and at 2.15 p.m. he was admitted to hospital.

Examination showed a boy of average physique; he was pale and had cold extremities, and was retching frequently. His pulse rate was 140, his blood pressure 90/60, and his respiration rate 35. For the next 10 hours retching and vomiting continued, and about every half-hour he brought up a little blood and sometimes shreds of mucosa. He was restless and quiet in turns, but was co-operative and quite aware of his surroundings. At 11 p.m. both plantar responses were extensor.

For the next 24 hours he remained drowsy and slept fitfully, but when awake was still very restless. Vomiting was not so frequent and no longer contained blood, and after midday it ceased altogether. His general condition improved and his extremities were warm, but both plantar responses remained extensor.

On February 4 alternating drowsiness and restlessness continued, but he appeared brighter in himself and took more interest in his surroundings. His temperature had gradually risen to 101° F. (38.3° C.), but thereafter was normal. Next day his behaviour was normal, plantar responses were flexor, and his bowels were opened for the first time. He was discharged home on February 8, and two weeks later was "very well."

Investigations.—February 2: 3.30 p.m., serum iron, 3.42 mg. per 100 ml. *February 8:* serum iron, 0.33 mg. Liver-function tests: thymol turbidity, 3 units; thymol flocculation, *nil*; cephalin-cholesterol, +; zinc sulphate, 3 units; alkaline phosphatase, 5.8 units; serum bilirubin, absent.

Case 3

At 5.30 p.m. on November 25, 1950, a boy aged 11 months was found vomiting on the floor and beside him was a packet of ferrous sulphate tablets: 13 tablets were missing. He was put to bed but continued to vomit, and the parents estimated that he had returned bits of tablets amounting to four to six whole tablets. He thus retained seven to nine tablets. By 6 p.m. he had turned very pale and lay very still, crying only when he wanted to vomit. The vomit was a clear brown fluid and did not contain blood. On admission to hospital at 6.40 p.m. he appeared pale, quiet, and rather drowsy, and there was slight cyanosis of the lips. At 7 p.m. the stomach was washed out; at first he cried and struggled, but towards the end of the procedure he lay very still. Physical examination at this stage showed a well-nourished infant with no abnormal signs. The pulse rate was 150, the respiration rate 35, and all deep reflexes were present and brisk. For the next three hours he remained very quiet and pale, and he vomited once. Thereafter his colour improved and he slept through the night. The next day he appeared lively and quite normal. He passed two loose black motions; the pulse rate dropped to 100 and the respiration rate to 24. On November 27 he remained well, and next day was discharged home.

Case 4

At 3.30 p.m. on February 6, 1951, a boy aged 20 months was noticed by his mother to be rather quiet; she thought he looked drowsy and ill, and she became alarmed when she could not rouse him properly. His sister said he had been eating some tablets, and investigation showed that a maximum of five ferrous sulphate tablets were missing. At 4.30 p.m. his mother gave him some salt and water to drink,

and he vomited several times, two tablets being returned. At 6.30 p.m. he was admitted to hospital and kept under observation for 24 hours, but he was cheerful and lively on arrival, and at no time showed any ill effects from the three tablets which he had retained. Treatment was considered unnecessary.

"Coroners' Cases"

Case 5

At 12.30 p.m. on August 20, 1950, a boy aged 12 months, who had always been healthy, was put to sleep in his parents' bed. At 1.15 p.m. he was found playing with an unknown quantity of ferrous sulphate tablets, and four or five were removed from his mouth. He was made to drink salt and water and he vomited, but no tablets were returned. He was given some castor oil and then ate his dinner. He did not appear to be upset. At about 2.45 p.m. he vomited a brown-coloured liquid containing six tablets. The family doctor prescribed a further dose of castor oil, and said that if the child became worse he should be sent to hospital. The child went to sleep but woke up again and vomited. He was cold and clammy. At 5 p.m. he was found to be dead.

Necropsy Report.—The body was that of a well-developed male infant. The nostrils, lips, and mouth were stained with a black liquid. The nasopharynx, trachea, and main bronchi contained much brownish-black semi-solid material, which was not frothy, and the lungs showed some mottling on their surfaces due to scattered areas of collapse. The stomach contained about 3 oz. (85 ml.) of thick black fluid and the mucous membrane was red and necrotic. A few scattered areas of congestion were present in the upper jejunum, and Peyer's patches of the entire small intestine were prominent. The large intestine contained black fluid but was otherwise normal. The liver was normal in size but rather pale. The heart, spleen, pancreas, kidneys, skull, and brain were normal macroscopically. The stomach contents gave a strongly positive dipyridyl reaction for iron.

Histology.—The stomach showed necrosis of the superficial layers of the mucosa. Prussian-blue staining revealed a mass of iron in the necrotic portions of the mucous membrane and smaller amounts being absorbed into the blood stream. The liver showed cloudy swelling of the cells of the centrilobular zone and a slight increase of polymorphonuclear leucocytes in the liver sinusoids.

Case 6

At 8.15 a.m. on September 10, 1947, a girl aged 19 months was found vomiting after taking 15 or 16 ferrous sulphate tablets. She was taken to hospital and there given salt and water, and she vomited again. The mother was told that there were no beds available, and she was sent to another hospital. Here she was told that the tablets were not poisonous and would do the child no harm. The child was retching now but not vomiting, and the mother was told to take her home and give her plenty of milk to drink. The mother was not satisfied and took the child to another doctor on the way home. He told her to give the child orange juice to drink and she would be all right. The mother then took the child home, put her in her cot, and went to make some orange juice. When she returned the child was dead. The time was 1 p.m., about four hours after the child had taken the tablets.

The necropsy showed intense congestion of the stomach, which contained blood and mucus, and the intestines were also congested. There was no vomit in the respiratory passages.

Case 7

At 9 a.m. on January 4, 1951, a boy aged 18 months started to cry and was given a cup of tea, whereupon he was violently sick and had diarrhoea. His mother found her box of ferrous sulphate tablets empty, and 44 tablets were missing. He was taken to hospital, where his stomach was washed out with 5 pints (2.8 litres) of fluid, flecks of blood and mucus being returned. Restoratives were administered, but he died. He was said to have been in a state of coma from 11 a.m., and he died at 2.30 p.m., five and a half hours after taking the tablets.

Necropsy showed acute dilatation of the right ventricle and congestion of the stomach and intestines, with, on analysis, substantial quantities of iron in the stomach contents. There was no vomit in the respiratory passages.

Case 8

One morning in September, 1949, a girl aged 14 months was found to have swallowed 44 ferrous sulphate tablets. The doctor was telephoned, but he said there was no danger. Later the child showed signs of distress and started to vomit, bringing up four tablets. The doctor was sent for and found a rapid pulse. He again said that there was no danger, but he called back later and prescribed castor-oil and kaolin. The child vomited and retched throughout the day, and in the evening was said to be semi-conscious and was put to sleep in her parents' room. During the early hours of the following morning the mother found her child to be dead, some 20 to 24 hours after taking the tablets.

At necropsy the stomach contained a quantity of black iron sulphide and showed intense congestion and oedema of its wall, with, in places, corrosion of the mucosa. There were "profound toxic changes in the liver."

The Clinical Picture

Excessive amounts of ferrous sulphate have a fairly constant effect on small children. Within the first hour even the mildest cases make their mothers apprehensive, for they look pale and ill and they generally vomit. At first the vomitus may contain unaltered tablets, and in the more severe cases it often contains small amounts of bright red blood by the third hour, and by this time the child presents the fully developed and characteristic

Summary of Case Records

Case No.	Age (Mths)	Sex	No. of Tablets Taken	No. of Tablets Returned and After How Long	No. of Tablets Retained	Treatment Before Admission	Time Before Admission	Treatment After Admission	Tachycardia	Early Pallor	Drowsiness	Vomiting	Haematemesis	Result	Time Before Death
1	21	F	75	21. 30 mins.	54	Salt water; gastric lavage	3 hours	Gastric lavage; bismuth carb; vitamin mixture	+	+	+	+	+	Recovered	—
2	23	M	33	4+. Few mins.	Less than 29	Salt water	3½ ,,	Bismuth carb.; I.V. glucose-saline; vitamin mixture	+	+	+	+	+	,,	—
3	11	M	13	4-6. Few mins	7–9	Nil	70 mins	Gastric lavage; bismuth carb.; vitamin mixture	+	+	+	+	—	,,	—
4	20	M	5	2. 1 hour	3	Salt water	3 hours	Nil	—	—	+	—	—	,,	—
5	12	M	?	6. 1½ hours	?	Salt water; castor oil	—	,,	?	?	+	+	—	Died	4 hours
6	19	F	15–16	None	15–16	Salt water	—	,,	?	?	?	+	—	,,	4 ,,
7	18	M	44	,,	44	Nil	?	Gastric lavage	?	?	+	+	—	,,	5½ ,,
8	14	F	44	4. ?	40	Castor oil; kaolin	—	Nil	+	?	?	+	—	,,	20–24 hours

This table is similar to that used by Thomson, but has been slightly amended

picture. Pallor, coldness, tachycardia, retching, vomiting, and drowsiness, together with restlessness, are almost constant. Of these vomiting and drowsy restlessness are the predominant features of the illness, and the length of time they continue depends on the number of tablets taken. Thus Case 4 was drowsy and looked ill for an hour or two after only three tablets, whereas Case 1, who retained 54 tablets, was semi-comatose for 24 hours and drowsy for four days, and it was eight days before she behaved normally.

Haematemesis in the first 12–24 hours is frequent, and, though it is an alarming symptom, it does not usually lead to an excessive loss of blood. Diarrhoea is uncommon, which is surprising when one considers the irritating effect of the tablets on the stomach. However, the small bowel escapes gross damage, and this may be due to the reaction of the alkaline intestinal juices converting the ferrous sulphate into insoluble iron compounds.

Increase in the respiratory rate was noticed in the first three cases, and in Case 1 the alteration was pronounced, the excursions were very shallow, and the rhythm was also irregular. These features have not been reported in other cases, though Somers (1947) noticed shallow breathing and an increased rate in experiments on rabbits. Two other cases have been reported with abnormal physical signs in the chest, but, in these, aspiration pneumonia was the probable cause.

Detailed physical examination adds little to what has been noted already. Abdominal distension is not present and tenderness is only infrequently found, and then is of slight degree. The central nervous system, apart from the altered mental state, has shown abnormal signs only in Case 2, in which bilateral extensor plantar responses were noticed on the first evening, and these reverted to normal on the fourth day as the child recovered.

A further feature that must be mentioned is a misleading period of clinical improvement preceding collapse which has been observed in some fatal cases during the second and third 12-hour periods. Thus Thomson's (1950) fourth case (reported fully by Prain, 1949) appeared quite well on the second day, but collapsed quite suddenly and died after 39 hours, whilst the other death in Thomson's series and Forbes's first case also followed this pattern.

Study of the case records of the eight deaths so far reported (including those in this series) reveal two critical periods in the illness. The first is after four to six hours, when three of the children died; the second is from 20–53 hours, and it includes the remaining five deaths. The significance of these periods is discussed later.

Mode of Action

It is clear from the necropsy findings in all cases that the stomach bears the brunt of the initial damage. It is reported as being oedematous and congested, with haemorrhagic and necrotic areas of variable extent which mainly involve the crests of the rugae. The small intestine is generally affected only in its proximal part, and then to a less degree than the stomach, and it shows congestion and perhaps oedema of the mucous membrane. The liver also shows gross changes, which vary from cloudy swelling to areas of necrosis, but the only other abnormalities that have been detected are cloudy swelling of the kidneys, heart, and pancreas, congestion of the spleen with necrosis of the Malpighian corpuscles, and congestion and patchy collapse of the lungs with aspiration pneumonia.

Prain suggested that liver failure is the cause of death in iron poisoning, but he admits that "the extent and degree of change in the liver seem scarcely sufficient to explain the fatal issue." It seems clear, too, that if liver failure is to be blamed for death then the histological changes are hardly comparable to those found in, for example, acute yellow atrophy, and in iron poisoning death occurs much quicker than is usual in that disease. For these reasons I would suggest that one must look elsewhere for the cause of death.

Serum iron estimations in Cases 1 and 2, between four and five hours after the tablets had been swallowed, showed levels of 3.3 and 3.4 mg. of iron per 100 ml., compared with the normal of 0.035–0.22 mg. per 100 ml. (Sven Dahl, 1948). These figures approximate almost exactly to the level, quoted by Slack and Wilkinson (1949), of 3.6 mg. per 100 ml. which is attained after an intravenous injection in adults of 200–300 mg. of an iron sucrose preparation. The symptoms reported to follow intravenous injections of iron include pallor, headache, vomiting, weakness, and collapse (Ramsey, 1950), and encephalopathy (Birch and Till, 1951), while Hurst (1931) and Napier (1936) recorded cases of presumed iron encephalopathy in adults after iron medication by mouth. There would seem to be a similarity here between the effects of iron given intravenously in adults and poisoning by ferrous sulphate in children, and I feel that iron poisoning results in a widespread interference with cell function, and that probably the most important organ involved is the brain. Histological proof of this is unfortunately lacking, for the available necropsy reports do not mention detailed examination of the brain.

I would suggest that the genesis of the illness is as follows. The initial vomiting, haematemesis, tachycardia, and collapse are due to the direct corrosive effect of the iron on the stomach. This causes considerable shock (as in other corrosive poisons), and it is this, together with the rising serum iron, which is the main cause of death in the first period (four to six hours). This stage of shock lasts for some 12–24 hours and then passes off, and it is its passing which causes the misleading improvement seen in some fatal cases during the second and third 12-hour periods. Concomitant with this process, and starting very soon after swallowing the tablets, iron is absorbed into the general circulation, and the serum iron may reach 15–100 times the normal level. The necropsy findings, though incomplete, make it reasonable to suppose that this amount of iron can cause profound cell dysfunction and that its effect will be general, though some tissues will be damaged more than others. The occasional untoward sequelae of intravenous injections of iron in adults and the not dissimilar type of illness observed in severe iron poisoning in children suggest that the central nervous system is deeply involved, and I feel that iron probably exerts its most important effect on the cells of this system and through this action causes death.

Treatment

Hitherto all reported cases have been treated "on general principles." That is to say, the stomach has been emptied either by an emetic, such as salt and water, or by lavage, and bicarbonate of soda or bismuth carbonate, or both, have been utilized to convert the soluble ferrous sulphate into insoluble iron compounds. These measures are both rational and simple, and were carried out on the cases in this series. In Case 5 six undissolved

tablets were vomited one and a half hours after the child had taken them, so, clearly, every effort should be made to empty the stomach in the first few hours; and it should be remembered that whole tablets will not pass through a stomach tube.

Dimercaprol ("B.A.L.") has been used on two occasions (Thomson, 1950; Roxburgh, 1949), but no claim of its efficacy has been made, and Edge and Somers (1948) have shown in experimental work on mice that when used either orally or intravenously it increases, rather than decreases, the toxic effects of ferrous sulphate. It was not used in this series.

Shortly after the admission of Case 1 to hospital Dr. A. L. Latner, of the Department of Biochemistry of this hospital, was called in consultation, and he made the following suggestions: (1) As iron might act as a heavy metal and combine with SH groupings and thereby interfere with oxidation, tocopherol, which acts as an antoxidant and appears to cut down cell oxidative requirements, should be prescribed. (2) Methionine should be given (*a*) as a source of SH groupings and (*b*) in an attempt to prevent the development of the fatty changes in the liver seen in some previous cases. (3) That the drowsiness and sudden death of previous cases might be due to some interference with the oxidative enzymes and with the utilization of the vitamin B complex. Also, as deficiency of certain members of this group is associated with fatty change in the liver, then members of the vitamin B complex should be given as well.

The outcome of these suggestions, later known as the "vitamin mixture," was prescribed, and the ultimate recovery of Case 1, who had taken a larger dose of iron than any other case previously described, encouraged us to use a similar regime in Cases 2 and 3, with equally satisfactory results. In Case 1 this treatment was started 22 hours after the tablets had been swallowed, and in Cases 2 and 3 it was started within a few hours of the child's arrival in hospital.

When Case 2 arrived in hospital, also having taken a very large dose of ferrous sulphate—98 gr. (6.5 g.)—though in this instance not all in tablet form, it was argued that if the 20–53-hour period was a critical one then it was important to see that the child entered it in as good general condition as possible. This child had haematemesis and was vomiting and thirsty, but he was not clinically dehydrated; however, he was given a slow intravenous infusion of 5% glucose-saline in order to maintain rather than correct the fluid balance. After this was carried out his condition improved, he had no further vomiting, and he made a rapid recovery.

The cases in this series and those described by Thomson (1947, 1950), Forbes (1947), and Roxburgh (1949), with a total mortality of 47% (17 cases, 8 deaths), give a more depressing view of ferrous sulphate poisoning than is justified, for there must be many mild cases which require little or no attention and, because they arouse no particular interest, are not reported. However, while these tablets remain in such common use it seems only too certain that there will be further cases, and for these I would suggest the following treatment:

1. Efforts to make the child vomit should be carried out immediately in the home, either by giving him salt and water to drink or by placing the fingers in his throat.

2. Gastric lavage with sodium bicarbonate solution as soon as possible: 10 oz. (285 ml.) of the solution should be left in the stomach.

3. Bismuth carbonate, 3 gr. (0.2 g.) should be given four-hourly.

4. Precautions should be taken against the inhalation of vomit.

5. Intravenous infusion of fluids should be considered. If shock is severe plasma should probably be used initially, otherwise 5% glucose-saline should be given to maintain fluid balance in severe cases.

6. The following vitamin mixture should be given:

Aneurin hydrochloride	10 mg.
Nicotinamide	30 „
Riboflavin	10 „
Tocopherol	15 „
Methionine	500 „

These amounts should be multiplied by the child's age in years and then divided into three daily doses.

With the exception of methionine all these substances can be given intramuscularly until vomiting stops; thereafter they can be given by mouth and often in tablet form, for these children have already shown an aptitude for swallowing tablets.

Prevention

Following, perhaps, on the suggestions of others who have written on this subject, the leading manufacturers of ferrous sulphate tablets have now printed in large letters on their packets that these tablets are dangerous to young children. This is the first step in prevention, but so far it is the only one that has been taken. Now, it seems irrational that some things which children are expected to take, such as cod-liver oil, remain unpalatable, whereas ferrous sulphate tablets, which are intended mainly for adult use, are made both attractive and sweet. Possibly adults would not take them if they were otherwise, and no suggestion would be acceptable which had this result; for these tablets play a vital part in maintaining the health of large numbers of the population, including the pregnant woman. For this reason I feel that the tablets should remain in their present form, but that some obstacle should be placed between the tablet and the child, and I would like to suggest that this could be done as follows:

The daily dose of three tablets should be wrapped separately and securely in small packets in the same way that sugar is sometimes supplied in hotels in packets containing two lumps. In the German Army Medical Service nearly all tablets were supplied in packets of five, and 10 of these units were fixed together, making bundles of 50. This was done presumably for ease in handling, but if it could be carried out as a general measure it should also be practicable in this instance. A glance at the case histories shows that almost all the children poisoned are under 2 years of age, and I feel that if members of this age group accidentally obtained tablets wrapped in the manner suggested they might play with them for a long time without opening the packet, and, even if they did free some of the pills, the speed at which they could swallow them in quantity would be very effectively reduced.

Summary

The clinical picture of iron poisoning in children is described in detail.

The effects of large doses of iron on children, and the genesis of the resulting illness, are discussed.

A new method of treatment is proposed.

A suggestion is made whereby further cases might be prevented.

My thanks are due to Professor Sir James Spence, Dr. Donald Court, and Dr. A. L. Latner for their helpful advice and encouragement: to Dr. J. Trepilus for the post-mortem report on Case 5; and to H.M. Coroners for Surrey, Liverpool, Louth, and Sunderland for their ready assistance and permission to use their reports in this paper.

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TWO CASES OF FERROUS SULPHATE POISONING

BY

JAMES THOMSON, M.B., F.R.C.P.

Paediatrician, Royal Infirmary, Dundee; Lecturer-in-Charge, Medical Diseases of Children, University of St. Andrews

After reading Dr. Gilbert Forbes's (1947) paper on poisoning with a preparation of iron, copper, and manganese I am prompted to record two further cases. It would seem that the condition may not be rare, and it is certainly one demanding increased precautions to prevent it.

Case 1

A 16-months-old girl was admitted to the Dundee Royal Infirmary on May 3, 1944. About 10.30 a.m. on that date she obtained a packet of 40 tablets, each containing ferrous sulphate exsic. gr. 3 (0.2 g.) copper sulphate gr. 1/25 (2.6 mg.), and manganese sulphate gr. 1/25. She swallowed all but one of these, and vomited almost at once, bringing up one tablet. Her mother gave her salt and water to drink, when about 12 more tablets were vomited. She thus retained about 26 of them. The child then became hot and drowsy and kept coughing and vomiting. The vomit contained mouthfuls of dark blood-stained materials.

She was admitted to hospital at 11.50 a.m. On examination she was rather pale, and was coughing and vomiting. The vomit was blood-stained. There was no rash or marked drowsiness. The temperature was 96° F. (35.6° C.), pulse 140, and respirations 24. The pulse was of slightly diminished volume. Her tongue was somewhat furred; the fauces were healthy. The abdomen was slightly distended. There was neither tenderness nor resistance on palpation. Nothing abnormal was noted in the central nervous system. Gastric lavage with a sodium bicarbonate solution was carried out, and brownish material was obtained. Some normal saline was left in the stomach. "Nepenthe" 1/2 min. (0.03 ml.) was given at once, and 2½ gr. (0.16 g.) of bismuth carbonate was given four-hourly.

In the evening she was breathing heavily, but was otherwise comfortable. There was no cyanosis during the night. No vomiting occurred, but one loose stool containing mucus and dark blood was passed. The temperature rose suddenly to 101.2° F. (38.4° C.) at 7.30 a.m. on the second day; her breathing became very difficult and she became cyanosed; she struggled, seemed to choke on inspiration, and died immediately.

Post-mortem Report (Dr. G. H. Smith).—A well-nourished female infant, rather cyanosed. Thorax: Heart and pericardium normal; both lungs show areas of patchy collapse and rather oedematous mucopurulent material in bronchi and trachea; no oedema of glottis. Abdomen: No free fluid in peritoneal cavity and no evidence of perforation. Stomach: Somewhat dilated. There is a large amount of brownish-black fluid present. The gastric mucosa is the seat of intense inflammatory change and there is a marked degree of necrosis and sloughing limited to the crests of the longitudinal rugae. Necrotic material from the stomach gives an intense iron reaction, the appearances being consistent with those due to corrosive poisoning. Numerous haemorrhagic points are apparent. Microscopical section shows necrosis of mucous membrane extending down to muscle. Intestines: Apart from a few patches of congestion in the upper jejunum, the intestines are healthy and the only contents present are black semi-fluid material resembling altered blood. Those contents are confined to the lower bowel, chiefly the pelvic colon. Liver: There is passive congestion. Neither hepatitis nor necrosis is present. Gall-bladder and pancreas: Healthy. Spleen: Healthy but congested. Kidneys and suprarenals: Healthy apart from venous engorgement. Ureter and bladder: Normal. Head: Brain and meninges healthy.

Case 2

A boy aged 2 years swallowed about 10 tablets similar to those mentioned above, about 9.30 a.m. on March 4, 1947. Approximately half an hour later he began to vomit green bile-stained fluid. Two tablets were returned whole. There was no haematemesis. His colour became waxy, but had improved before admission to hospital the same day at 2.5 p.m. He was very thirsty. A stool passed later in the day was dark and gave the guaiac reaction for blood.

On admission he was rather flushed, listless, and drowsy. His pulse was regular and of good volume. There was neither tenderness nor abnormal resistance over his abdomen. Treatment consisted of mist. magnesium hydroxide and bismuth, along with glucose and saline, later changed to milk and subsequently to milk diet. By March 6 the vomiting had ceased, and on the 9th the stool was normal, with no blood. On the 13th the child was very well, but the stool was dark and the guaiac test positive. He had had no meat since admission. On the 16th the stool was again dark, and on the 18th he was well and there was no melaena.

Comment

Ferrous sulphate poisoning is a serious risk in view of the widespread use of this type of tablet. It is important that they should be kept in a place where children cannot obtain them, and that their potential toxicity should be realized by the profession.

The first patient died after consuming about 26 tablets. The second survived after retaining about eight. BAL, which was referred to in an annotation (*Journal*, March 22, 1947, p. 386), was not available for the former case in 1944 and was not required in the latter one.

An old name for ferrous sulphate is green vitriol.

It is important to realize that these cases and the two quoted by Forbes have all resulted from gross overdosage.

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FERROUS SULPHATE POISONING

ITS INCIDENCE, SYMPTOMATOLOGY, TREATMENT, AND PREVENTION

BY

JAMES THOMSON, M.B., Ch.B., F.R.C.P.Ed.

Paediatrician, Royal Infirmary, Dundee; Lecturer-in-Charge, Medical Diseases of Children, University of St. Andrews

A pathological report on a case of ferrous sulphate poisoning admitted to my ward Prain (1949) describes the lesions, with their probable toxic significance, and the risk that these attractively coloured pills entail when young children have access to them. An annotation in the *British Medical Journal* (1949) emphasizes this risk and points out that all the recorded cases have occurred in Scotland and the northern half of England. Another case has in fact occurred in Surrey (*Surrey Comet*, 1949). It has accordingly seemed worth while to review the experience of this one hospital unit, which serves Dundee, with a population of 182,000. All the cases came from Dundee and no others are known to have been admitted elsewhere in the city. The first case occurred on May 3, 1944. This is the earliest case known to have been recorded in modern times. It was fatal, and along with the second case, which occurred on March 4, 1947, has already been fully reported (Thomson, 1947).

Six cases have occurred in the period from May 3, 1944, to October 2, 1949, and have all been under my care in Dundee Royal Infirmary. All took similar tablets containing ferrous sulphate exsic. 3 gr. (0.2 g.), copper sulphate 1/25 gr. (2.6 mg.), and manganese sulphate 1/25 gr. (tab. ferrous sulph. co., *B.P.C.*).

Case 1 (Summary of Report)

On May 3, 1944, a 16-months-old girl took 40 ferrous sulph. co. tablets from a paper packet and swallowed 39 of them at 10.30 a.m. She was given salt and water to drink and returned 13. Thus 26 were retained. She became hot and drowsy, and kept on coughing and vomiting. The vomit contained blood-stained material. On admission at 11.50 a.m. she was drowsy, pale, and still coughing and vomiting. The temperature was 96° F. (35.6° C.), pulse 140, and respiration 24. The pulse was of slightly diminished volume. The abdomen was slightly distended, but there was neither tenderness nor resistance on palpation. In the evening she was breathing heavily but was otherwise comfortable. No further vomiting occurred, but one loose stool containing mucus and dark blood was passed. At 7.30 a.m. on May 4 (21 hours after taking the tablets) her temperature rose suddenly, breathing became difficult, and the child became cyanosed. She appeared to choke on inspiration and died at once.

At post-mortem examination Dr. G. H. Smith found intense inflammatory change in the gastric mucosa and a marked degree of necrosis and sloughing limited to the crests of the longitudinal rugae. The necrosis extended down to muscle. In contrast to the stomach there were only a few patches of congestion in the upper jejunum. The intestine was otherwise healthy.

Treatment.—She received salt and water to drink before admission, and on admission gastric lavage with a sodium bicarbonate solution was carried out. Normal saline was left in the stomach, and 2½ gr. (0.16 g.) of bismuth carbonate was given four-hourly, and sodium bicarbonate 15 gr. (1 g.) at 8 p.m. She was also given glucose and orange juice, and later diluted milk.

This child died after retaining about 26 pills in spite of what, in view of subsequent investigations (Somers, 1947), seems to have been the correct treatment with bicarbonate solution to dilute the corrosive iron and also to reduce its solubility by altering it to the less irritant ferrous carbonate.

Case 2 (Summary)

On March 4, 1947, at about 9.30 a.m., a boy aged 2 swallowed about 10 tablets similar to those in Case 1. Approximately half an hour later he began to vomit, and two tablets were returned whole. There was no haematemesis. His appearance became "waxy," but improved before he was admitted at 2.5 p.m. the same day. On admission he was flushed and drowsy, and there was neither abdominal tenderness nor abnormal resistance on palpation. A stool passed later in the day gave a positive guaiac reaction. Vomiting ceased by March 6 and the stool was normal by the 9th with no blood. On the 13th the guaiac test was again positive; no meat had been given. Further recovery was uneventful.

Treatment.—He was given ½ oz. (14 ml.) of mist. magnesium hydroxide on admission and bismuth bicarbonate 2½ gr. four-hourly along with glucose and water, later changed to milk and subsequently to milk diet.

Case 3

On July 6, 1947, a girl aged 4½ years swallowed about 24 tablets. An hour later, according to her mother, she vomited about 20 of them. On admission she was slightly pale. Her abdomen was normal on palpation. She received 2½ gr. of bismuth carbonate four-hourly, but never exhibited any symptoms of illness except the vomiting before admission. According to the history she returned 20 tablets in recognizable shape one hour after swallowing them.

Case 4

On February 8, 1948, a girl aged 11 months was admitted under my care. A full report of this case has been published by Prain (1949). A summary of the case is given here.

At 7.30 p.m., while playing with a box of these tablets prescribed for her mother, she swallowed an unknown quantity of them and became drowsy and vomited. On admission at 9.30 p.m. she was pale and drowsy; the abdomen was soft and not tender. She vomited altered and bright-red blood until 10 p.m., when vomiting ceased. Next day she seemed well and played with her toys. She passed two tarry stools. But on the morning of the 10th she became cyanosed and had moist accompaniments at both bases. She died 39 hours after taking the pills.

On post-mortem examination extreme congestion of the mucous membrane was found; but the necrosis, though locally severe, was not so striking as in Case 1. The small intestine shows a few areas of congestion less marked than in the stomach.

Treatment.—This consisted of gastric lavage with sodium bicarbonate in water, also bismuth bicarbonate 2½ gr. in mist. cretae four-hourly.

Case 5

On July 14, 1949, a boy aged 19 months took some tablets off a shelf and was found eating them. He probably ate about 10. Before admission he was given syrup of figs and then salt and water, after which he vomited brown fluid several times. There was no red blood in his vomit. He then went to sleep, and was admitted 1½ hours after taking the tablets. A stool examined on the 16th gave positive reactions to Kastle-Meyer and benzidine tests for blood. Recovery was uneventful.

Treatment.—Gastric lavage with sodium bicarbonate produced clear fluid with streaks of blood-stained mucus in it. On the 14th dimercaprol, 0.5 ml., was given four-hourly for a few doses, but was stopped on the 15th.

Case 6

On October 2, 1949, a boy aged 2½ years was discovered at 8 p.m. with his mouth full of tablets which had been supplied to his mother. The quantity he ate is not accurately known, but was probably between 10 and 20. He was given syrup of figs at home, and vomited at 8.15 p.m.; subsequently he vomited five more times. The first five vomits contained "white bits and green stain." The last vomit contained only "water." He complained of a sore stomach after swallowing the pills, and was drowsy at 9.30 p.m. He was admitted at 11 p.m. awake but sleepy. His abdomen was soft and not tender. Gastric

	Age and Sex	Tablets Taken	Tablets Returned After Interval of	Tablets Retained	Treatment Before Admission	Time Before Admission	Treatment After Admission	Early Pallor	Drowsiness	Vomiting	Abdominal Pain	Haematemesis	Guaiac or Kastle-Meyer or Benzidine Test	Sudden Collapse	Result
1	16/12 M	39	13; less than 1 hour	26	Salt and water	1 hr. 20 mins.	Soda bicarb. lavage, bismuth carb.	+	+	+	−	+	+	+	Died
2	2 yrs. M	10	2; ½ hour	8	Water freely	4½ hrs.	Mist. magnes. hydrox. and bismuth carb.	+	+	+	−	−	+	−	Recovered
3	4½ yrs. F	24 ±	20; 1 hour	4	None	?	Bismuth carb.	+	+	+	−	+	−	−	
4	11/12 F	?	None	?	None	2 hrs.	Soda bicarb. lavage, bismuth bicarb., mist. cretae	+	+	+	−	+	+	+	Died
5	19/12 M	10 ±	None	10 ±	Syrup of figs; salt and water emesis	1½ hrs.	Soda bicarb. lavage, dimercaprol	−	+	+	−	+	+	−	Recovered
6	2½ yrs. M	10–20	Fragments, less than 3 hours	?	Syrup of figs	3 hrs.	Gastric lavage ½ oz. mag. sulph.	−	+	+	+	−	−	−	"

lavage was carried out with saline, and ½ oz. (15 g.) of magnesium sulphate in solution was left in the stomach. On the 3rd and 4th he passed two loose dark stools each day. The guaiac and Kastle-Meyer tests were negative. His further recovery was uneventful.

Discussion

Textbooks differ in their statements about tests to distinguish iron from haemoglobin. Tests showed that the Kastle-Meyer and benzidine tests give a brown deposit with iron solutions quite distinct from the colour reactions with haemoglobin. The guaiac test, while distinguishing between the two, does not give such obviously contrasting reactions.

So far as is known no other cases have occurred in Dundee in this period of 5 years and 5 months.

It thus appears that the condition is not rare. No explanation for the known cases occurring in the more northern parts of Britain can be offered except the accident of reporting.

The clinical picture of these cases is pallor at an early stage, followed by drowsiness, and vomiting early but ceasing after a few hours. Abdominal pain was noted in only one case, while none had abdominal tenderness or abnormal abdominal resistance. Haematemesis may be severe and early or not be clinically evident. Blood is probably present in the stools in most cases. A most misleading period of well-being, even playfulness, was seen in Cases 1 and 4 and in Forbes's (1947) first case. This was followed by fatal collapse. An important point is that whole tablets or fragments of them may persist in the stomach for at least one hour. Emesis in addition to lavage is therefore important for prompt elimination of any iron still in pill form.

The treatment given to Case 1, and subsequently experimentally justified by Somers, seems to have been appropriate—namely, gastric lavage with an aqueous bicarbonate solution to convert as much as possible of the corrosive ferrous sulphate to the much less irritant ferrous carbonate and to dilute it. Also to get rid of pills by emesis, which has been successfully done half an hour to one hour after ingestion; then to protect the mucosal lesions by bismuth. This seems to be the rational method of attempting to prevent the delayed sudden fatal collapse occurring after a deceptive period of improvement. This collapse is possibly due to toxic absorption from damaged mucous membrane affecting the liver (Prain, 1949). Dimercaprol, although tried, did not appear to help, and it is now suggested that it may be harmful (Edge and Somers, 1948).

It is abundantly clear that prevention of this accidental poisoning is important and urgent. The makers of "fersolate" tablets have now added a warning: "Excessive doses of iron can be dangerous. Do not leave the tablets within reach of young children, who may eat them as sweets with harmful results." They also supply the tablets in a screw-topped container which would baffle the younger children. Welfare and antenatal clinics in particular should review their methods of issuing the useful ferrous sulphate tablets to adults. They should never be issued in paper packets or a carton with a loose-fitting lid. However, children on occasion will exercise much perverse ingenuity. One boy, aged 6½, obtained and swallowed a poisonous quantity of pills containing iron, arsenic, aloin, strychnine, and capsicum by raiding a drawer in his friend's aunt's house.

The hope expressed in an annotation in the *British Medical Journal* (1947) that recognition of the danger would secure adequate precautions has not been realized. It would seem to be urgent that administrative action should be taken now to prevent these useful tablets being dispensed in unsafe containers, and that adequate warning be given on the containers of their potential danger to children when ingested in excessive quantity, as happens when children regard them as sweets. They should also be stored in a really safe place. It is considered insufficient to make them a less attractive colour, as the pleasant taste of the coating would still appeal to children and cause them to continue to suck and swallow more pills. I wish to oppose the suggestion (*Lancet*, 1949) that these very useful pills should be withheld from adults where there is a young family.

Summary

Six cases of ferrous sulphate poisoning are reported occurring in 5 years and 5 months in a town with a population of 182,000. Two patients died and four recovered. Early pallor, drowsiness, vomiting, haematemesis, and later melaena were the main symptoms. Fatal collapse may follow apparent recovery. The treatment given is described and discussed. An urgent plea is made for administrative action to secure really safe packing and storing of these pills when dispensed. Ferrous sulphate is so valuable when properly employed that it is important to prevent undeserved unpopularity due to the gross abuse of it. Gross abuse is a fair description of its use in the present series of cases.

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COMPARATIVE TOXICOLOGY OF IRON COMPOUNDS

By LAWRENCE C. WEAVER, PH.D.
ASSOCIATE DIRECTOR OF PHARMACEUTICAL RESEARCH

ROBERT W. GARDNER, PH.D.*
PHARMACOLOGIST

VERNE B. GOPINGER, D.V.M., PH.D.
DIRECTOR OF PATHOLOGY

AND

CARL A. BURGE, M.D., PH.D.†
VICE-PRESIDENT, RESEARCH

From the Departments of Pharmacology and Pathology, Research Center, Pitman-Moore Company, Indianapolis, Indiana)

THE great number of iron compounds available for the treatment of iron deficiency anemia is evidence of the incidence and seriousness of this clinical problem and the lack of a truly acceptable preparation for therapy. The principal problems encountered in oral administration of iron preparations involve gastrointestinal distress and, more importantly, serious and often fatal intoxications in children.

This report is concerned with the animal toxicities of a series of ferric iron-carbohydrate complexes and ferrous salts. The prep-

Materials and Methods. The carbohydrate complex [illegible] stabilized with [illegible] (Fe) in the ferric form [illegible] with a polysaccharide of [illegible] and molecular weight (30,000). In physical properties, it is a free-flowing, amorphous, brown powder-like [illegible] of [illegible] tions from [illegible] 5.0). It forms a dark brown colloidal solution that is stable at pH 4 to 11 and to heat.

Other compounds used were exsiccated ferrous sulfate (Fe 29.7%); ferrous gluconate (Fe 11.6%); ferrous fumarate§ (Fe 32.9%); ferric choline citrate (Fe 12.0%); an iron polysaccharide complex‖, which contains 20 mg. of trivalent iron per milliliter (given by intravenous injection), and tablets of ferroglycine sulfate complex, available commercially as Ferronord, which were used in pulverized form.

ACUTE TOXICITY IN MICE. The compounds were administered as aqueous solutions where possible, otherwise as fine suspensions. Groups of 10 or more male albino Swiss-Webster mice were given the compounds (Table 1) intravenously (i.v.), intraperitoneally (i.p.), or intragastrically (i.g.). The rate of i.v. injections was 0.01 ml. per second. The animals were observed closely for several hours following injection, and the LD_{50} and 95% confidence limits were determined at the end of 24 hours by the method of Litchfield and Wilcoxon[3]. The animals receiving iron-carbohydrate complex and ferrous sulfate were observed for a period of 7 days following injection and any delayed manifestations of toxicity were recorded. If any deaths occurred after 24 hours, the LD_{50} was recalcu-

[illegible] Department of [illegible]ology, Indiana University Medical Center, [illegible]s, Indiana.
*Present address: Wm. S. Merrell Co., Cincinnati, Ohio.
†Present address: Pitman-Moore Co.
‡Supplied by Edwin P. Hallen, Mallinckrodt Chemical Works, St. Louis, Mo.
§Supplied by [illegible] Pharmaceutical Products, Inc., Worcester, Mass.

[illegible] end of the 7-day observation period. Local tissue reactions in response to iron-carbohydrate complex and ferrous sulfate were evaluated by the use of female albino Swiss-Webster, black female bDF-1 and C-57 and female fawn DBA-2 mice.

ACUTE TOXICITY IN RATS. Male (250 to 350 gm.) and female (150 to 250 gm.) Harlan-Wistar rats in mixed groups of 6 received the iron compounds i.g. in attempts to determine 24-hour LD_{50} in this species.

TOXICITY IN DOGS. Mongrel dogs, unselected as to sex, were used in all acute toxicity studies. Acute, rapid, i.v. injections were made and the LD_{50} determined at 24 hours. In subacute toxicity tests, dogs received iron-carbohydrate complex or ferrous sulfate in gelatin capsules twice daily for approximately one month. The total dose of ferrous sulfate was 0.5 gm. per day (3 dogs) and the total daily doses of iron-carbohydrate complex were 0.5 gm. (3 dogs), 1.0 gm. (3 dogs), and 2.0 gm. (3 dogs). These animals were observed closely for emesis and other outward signs of toxicity. At the end of the test period the dogs were killed (pentobarbital sodium solution intracardially and exsanguination) and subjected to extensive histopathologic studies.

PATHOLOGIC STUDIES. Blood samples from all dogs used in subacute toxicity tests were taken for routine hematologic studies, and thorough necropsy examinations were made for gross lesions.

Fresh specimens of liver, spleen, bone marrow, stomach, small intestine, large intestine, brain, kidneys, adrenal glands, mesenteric lymph nodes, thyroid, and myocardium were fixed in formalin, processed, and sections (about 6 μ) of all tissues were stained with azure-eosin. In addition, sections of liver, spleen, and bone marrow were stained by a modified Gomori's method for iron.

EMETIC STUDIES. The various iron compounds were given in suspension (ferrous sulfate), in solution (iron-carbohydrate complex), or in gelatin capsules. The dogs were not fasted and no dog was used more than once in these studies.

Results. The results in mice are summarized in Table 1. Since the iron content of the compounds varies considerably, comparisons were made on the basis of actual iron content. The iron polysaccharide complex was the least toxic [illegible] and iron-carbohydrate complex was next. The other compounds tested were 2 to 4 times as toxic as the iron-carbohydrate complex. The iron-carbohydrate complex was the least toxic of the compounds studied i.p. in mice, though not significantly less toxic than the iron polysaccharide complex. It had only 1/10 the toxicity of ferrous sulfate. Intragastrically, iron-carbohydrate complex was at least 6 times less toxic than any of the other compounds tested. The volumes necessary made it impractical to attempt to determine the i.g. toxicity of the iron polysaccharide complex. There was no significant difference between the 1- and 7-day toxicities for either iron-carbohydrate complex or ferrous sulfate for the i.v. and i.g. routes in mice.

In Swiss-Webster male mice the i.g. LD_{50} was 1025 mg. per kg. for ferrous sulfate. The same dose in female mice of different strains (10 mice in each test) produced the following percentages of deaths: Swiss-Webster, 70; BDF-1, 70; and C-57, 90. The i.g. LD_{50} of iron-carbohydrate complex in male Swiss-Webster was >8000 mg. per kg. The same dose produced no deaths in female Swiss-Webster, BDF-1, C-57 and DBA-2. Thus, there is no evidence of sex or strain differences in these very limited studies.

Results of acute toxicity studies in rats and dogs are summarized in Table 2. None of the compounds are very toxic following i.g. administration to rats and evidently there are only slight differences in the lethal effects of ferrous sulfate, ferrous gluconate, and ferroglycine sulfate complex. There was no significant difference between the 1- and 7-day LD_{50} for ferrous sulfate and iron-carbohydrate complex in rats.

Following i.v. administration in dogs ferric choline citrate and ferrous sulfate were the most toxic of the compounds tested. Ferrous gluconate and

TABLE 1. ACUTE TOXICITIES OF IRON COMPOUNDS IN MICE

LD_{50} (19-20 Confidence Limits), mg./kg.

Compound	*% Fe*	*No. mice*	*i.v. route As salt*	*As Fe*	*No. mice*	*i.p. route As salt*	*As Fe*	*No. mice*	*i.g. route As salt*	*As Fe*
Iron-carbohydrate complex	18.8	40	175 (158-194)	85	30	980 (831-1151)	478.2	30	>8000	>3904
Ferrous sulfate	29.72	55	112 (105-120)	33	105	137 (122-154)	40.7	40	1025 (802-1311)	305
Ferrous gluconate	11.58	55	199 (182-218)	23	30	460 (94-272)	18.5	100	3950 (3543-4404)	457.4
Ferrous fumarate	32.87	--	--	--	10	480 (410-562)	157.8	70	1570 (1353-1821)	516.1
Ferroglycine sulfate complex	15.87	--	--	--	80	365 (341-391)	57.9	50	1940 (1516-2488)	307.9
Ferric choline citrate	12.02	70	210 (192-229)	25	10	151 (120-190)	18.1	40	5500 (4297-7040)	661.1
Iron polysaccharide complex	2.0	30	--	170 (147-197)	50	--	318 (288-426)	--	--	--

TABLE 2. ACUTE TOXICITIES OF IRON COMPOUNDS IN RATS AND DOGS

LD_{50} (19-20 Confidence Limits)

Compound	*% Fe*	*No. rats*	*i.v. route As salt*	*As Fe*	*No. dogs*	*i.p. route As salt*	*As Fe*
Iron-carbohydrate complex	18.8	10	8000	>3904	18	94 (78-113)	45.9
Ferrous sulfate	29.7	24	2625 (2323-2956)	780	16	79 (71-88)	23.5
Ferrous gluconate	11.6	24	7450 (6644-8351)	865	9	>400	>46.4
Ferrous fumarate	32.9	24	>7080	>2329	—	—	—
Ferroglycine sulfate complex	15.9	24	5590 (4451-7014)	891	—	—	—
Ferric choline citrate	12.0	12	>8000	960	17	140 (103-190)	16.8
Iron polysaccharide complex	2.0	--			3		>40

iron-carbohydrate complex were the least toxic. The iron polysaccharide complex was given in a dose of 40 mg. per kg. to 3 dogs without lethal effects. Because of the fixed concentration of the solution, the volume necessary for higher dosages was too great to be practical.

weights of 6 dogs on the 2 gm. per day dose of iron-carbohydrate complex showed a 0.1 kg rise as the maximum change. Emesis occurred once in the 12 dogs during the study with iron-carbohydrate complex and this was at the dose of 0.5 gm. per day. In contrast, emesis occurred 14 times during

TABLE 3. EMETIC EFFECTS OF IRON COMPOUNDS IN DOGS

Compound	*Form*	*Dose mg./kg.*		*No. Vomiting/ Total No.*	*% Emesis*
		As salt	*As Fe*		
Ferrous sulfate	Suspension	96	28.8 (10.5–42.6)	—	ED_{50}*
	Capsule	62	18.6 (15.3–22.8)	—	ED_{50}
Iron-carbohydrate complex	Solution	600	293	0/4	0
		900	439	0/7	0
	Capsule	300	146	0/2	0
		600	293	1/4	25
		900	439	0/10	0
Ferrous gluconate	Capsule	400	46	2/5	40
		800	93	5/5	100
Ferrous fumarate	Capsule	800	263	4/6	67
Ferroglycine sulfate complex	Capsule	200	32	2/4	50
		400	64	2/2	100
		800	127	2/2	100
Ferric choline citrate	Capsule	800	96	1/5	20
		1200	144	2/6	33

*ED_{50} = Emetic dose for 50% of dogs.

Emetic responses to the various iron compounds in dogs are presented in Table 3. Iron-carbohydrate complex produced less gastrointestinal distress as indicated by emesis than any of the other compounds. Ferrous sulfate and ferroglycine sulfate complex were the most emetic in this study.

In the subacute toxicity studies average weights of dogs given ferrous sulfate and those given iron-carbohydrate complex at the two lower dosage levels decreased the first week and then remained constant or were regained. The changes ranged between 0.6 and 0.8 kg. for the 3 groups. The average

the same period in the 5 dogs given ferrous sulfate. No other gross signs of toxicity were observed.

No gross lesions or microscopic lesions suggestive of iron toxicity were observed in any of the dogs regardless of the compound or dosage level. No significant differences in stained iron content were apparent in the spleen, liver, and bone marrow at any dosage level of either ferrous sulfate or iron-carbohydrate complex. The total red blood cell count and hemoglobin levels were within the normal range in each dog.

Discussion. Studies of ferrous sulfate.

ferrous gluconate and ferrous fumarate in rodents gave results comparable to those reported by other investigators (Table 4). The consistently lower toxicity observed in our studies for these compounds in rats might be explained by the fact that our studies were done in older animals that had free access to food except during the period of testing; no sex differences were observed in these studies or in the studies conducted in mice.

stained iron content in the spleen, liver, or bone marrow from lower dosages or from ferrous sulfate. This suggests that even high doses of iron-carbohydrate complex may be tolerated without serious side effects.

Summary. 1. Studies in mice indicate that iron-carbohydrate complex is less toxic than ferrous sulfate, ferrous gluconate, ferrous fumarate, ferroglycine sulfate complex, ferric choline citrate and iron polysaccharide complex by

TABLE 4. ACUTE TOXICITIES OF IRON COMPOUNDS IN MICE AND RATS

	LD$_{50}$, mg. Fe/kg.			
Compound	*mice, i.g.*	*mice i.v.*	*rats, i.g.*	*Reference*
Ferrous sulfate	305	33	780	
	306	13	298	Hoppe *et al.*[3]
	230	11	344	Berenbaum *et al.*[1]
				Nissim[6]
				Keith[4]
	900	14		Somers[7]
				Edge *et al.*[2]
Ferrous gluconate	157	23	865	
	429	13	518	Hoppe *et al.*[3]
	320			Berenbaum *et al.*[1]
Ferrous fumarate	516		>2329	
	630		580	Berenbaum *et al.*[1]

Iron-carbohydrate complex showed a low order of toxicity in 3 species of laboratory animals. With one exception none of the 6 iron compounds tested was less toxic than iron-carbohydrate complex; the iron polysaccharide complex was less toxic by the i.v. route in mice. Further, gastric intolerance as indicated by emesis was very much less for iron-carbohydrate complex than for the other 5 compounds. In doses equivalent to 1 gm. of elemental iron daily for a month, gross or microscopic changes were not found in dogs. Large doses of iron-carbohydrate complex (2 gm. per day) produced no significant differences in the oral and intraperitoneal routes. The iron polysaccharide complex by the intravenous route was the least toxic and iron-carbohydrate complex was next least toxic.

2. None of the compounds tested was less toxic than iron-carbohydrate complex by the oral route in rats or the intravenous route in dogs.

3. Iron-carbohydrate complex produced the least gastrointestinal irritation as indicated by emesis in the dog.

4. Doses of iron-carbohydrate complex equivalent to 1 gm. elemental iron per day for a month failed to produce local irritation or systemic alterations in dogs.

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Ferrous sulphate
hemodynamics
iron poisoning
shock

Studies in Acute Iron Poisoning III. The Hemodynamic Alterations in Acute Experimental Iron Poisoning

C. F. Whitten[14], Y. C. Chen and G. W. Gibson

Department of Pediatrics, Wayne State University School of Medicine, and the Children's Hospital of Michigan, Detroit, Michigan, USA

Extract

One hour after administration of a lethal dose of ferrous sulfate into the intestine, 10 dogs sustained a sharp decline in cardiac output (mean 57%), a lesser reduction of arterial blood pressure (mean 17%), and a marked elevation of total peripheral resistance (mean 100%). Thereafter, the cardiac output decreased more rapidly than did blood pressure. Total peripheral resistance remained elevated until death.

Only minimal reduction in total blood volume (mean 9%) was observed one hour after challenge, but the plasma volume was significantly reduced (24%). Largely as a result of the reduction in plasma volume, the total blood volume was 70% of the baseline level just before death.

Speculation

It is unlikely that the simple early restoration of blood volume through plasma or plasma expanders would significantly alter the mortality in dogs poisoned with an LD_{100} dose of iron. This approach, however, could enhance the effectiveness of therapy with the promising chelating agent desferrioxamine. Desferrioxamine promotes the excretion of iron via the kidney. Early correction of blood volume deficits might maintain renal function for a sufficient length of time to permit excretion of critical quantities of iron.

Introduction

The pathophysiologic disturbances in acute iron poisoning are due to the effect of absorbed iron [7]. Shock the major alteration induced by the absorbed iron 10]. This study was undertaken to determine the sequence and relative importance of the hemodynamic events which lead to the development of shock, since these aspects of acute iron poisoning have not been adequately investigated.

Methods and Materials

The subjects, 10 female mongrel dogs, were given water, but no food, for 24 hours prior to the study. Following anesthetization with sodium pentobarbital (30 mg kg body weight), iron poisoning was induced by the administration of an LD_{100} dose of elemental iron, 225 mg/kg given as a 25% aqueous ferrous sulfate solution [10]. The solution was injected into the duodenum through a midline abdominal incision (7 dogs) or was

instilled in the stomach through a Blakemore tube (3 dogs). The duodenal balloon of the Blakemore tube was inflated to prevent the loss of iron through vomiting. An endotracheal tube was inserted in the trachea to prevent aspiration. Urine was collected through an indwelling catheter in the bladder.

Polyethylene catheters were placed in the abdominal aorta (via a femoral artery), the inferior vena cava (via a femoral vein), and the portal vein (via a splenic vein) to permit constant monitoring of systemic blood pressure (BP), central venous, and portal vein pressures. These were measured and recorded with Statham transducers and a Gilson polygraph. Catheters were inserted via the other femoral artery and vein into the abdominal aorta and inferior vena cava to measure cardiac output. For each determination, 0.5–3.0 mg of cardiogreen (5 mg/ml) was injected into the inferior vena cava. Blood was withdrawn from the aorta through a Waters densitometer by a Harvard constant infusion withdrawal pump, and dye curves were recorded on a Sargent recorder. The output was calculated according to the formula of HETZEL [5]. The total peripheral resistance was calculated from the formula [3]:

$$\text{Peripheral resistance units (PRU)} = \frac{\text{mean arterial BP (mm Hg)}}{\text{cardiac output (ml/s)}}$$

Plasma volume and red cell mass were determined simultaneously and independently by a double-labeling radioisotope dilution technique [2]. Plasma volume was determined by injecting 1 ml of human serum albumin labeled with 2.0 microcuries of I^{125}. The red cell mass was determined by injecting 1 ml of dog blood containing red cells labeled with 30 μc of Cr^{51}. A single blood sample was withdrawn 15–20 minutes after concomitant administration of the mixture of labeled substances. The measurements were made by use of a Volumetron, a semi-automatic electronic blood volume computer [12].

Arterial pH was measured with a Radiometer pH meter using a Sanz type electrode; for this, blood was collected anaerobically and placed on ice until analyzed. Concentrations of iron in serum and urine were determined by the methods of GIBBONS [10]. Microhematocrit determinations were made with an International microhematocrit centrifuge.

Approximately 20 ml of blood was removed each hour for making measurements, and this was replaced immediately with normal dog blood.

Results

An increase in serum iron concentration, a decrease in arterial pH, and an increase in hematocrit were consistently present at one hour after challenge. Progressive changes in the same direction usually continued until death occurred at 3 to 9 ½ hours (table I). Severe oliguria developed in 8 of the 10 dogs. These features were consistent with the course of acute iron poisoning as previously described [7, 8, 10].

Cardiac Output, Total Peripheral Resistance, and Mean Arterial BP

At one hour after the injection of iron there was a sharp decline in cardiac output (mean 57 %), a less severe reduction in mean arterial BP (mean 17 % with virtually no change in 3 dogs), and a marked elevation in total peripheral resistance (mean 100 %) (tables III and IV).

Subsequently, the fall in BP did not parallel the decrease in cardiac output and moderately high BP readings were obtained until approximately one-half hour before death. The last values for cardiac output measured approximately one hour before death were less than 25 % of the prechallenge level. Total peripheral resistance was elevated throughout the period of observation.

Central Venous and Portal Vein Pressures

Alterations in central venous pressure at one hour (8 animals) were variable. In four animals, no change occurred; in others, there was a decrease of 23–63 %. Subsequently, the former group showed little change, but the pressure in the latter group returned approximately to baseline levels.

The changes in portal vein pressure (6 animals were also inconsistent. In five animals, there was a significant rise at one hour (mean 36 %) and a subsequent return to near normal levels at the time of the last determinations. In one animal, there was a slight fall at one hour. The final determinations were slightly higher than the baseline values in two and slightly lower in four dogs (tables III and IV).

Plasma Volume, Red Cell Mass, and Total Blood Volume

When measured at one hour, plasma volume decreased (mean 24 %). Serial determinations revealed a progressive decline, and the last analysis showed a volume less than 50 % of the prechallenge level in 7 of the 10 animals. The changes in red cell mass were less marked. There was a slight to moderate increase at one hour and subsequently a return to values similar to or below control levels. With one exception, the algebraic sum of plasma volume and red cell mass (total blood volume) was moderately decreased at one hour (mean 9 %). Serial determinations indicated further reductions and the average preterminal deficit was approximately 30 %.

Table I. Selected data on animals subjected to acute iron poisoning

Dog No.	Weight kg	Time of death h	Urine[1] ml	Iron[2] mg	Serum iron mg %								Arterial pH								Hematocrit (vol. %)							
					Hours after injection of iron																							
					0	1	2	3	4	5	6	8	0	1	2	3	6	5	6	8	.0	1	2	3	4	5	6	8
1	20.7	6 ¼			0.13	7.6	9.4	11.1	11.1				7.28	7.13	7.06		7.07	6.93			45	64	70		72	73		
2	16.0	3	1		0.12	2.4		5.3					7.30	7.22		6.95					43	55		55				
3	12.5	5 ½	37	1.1	0.08	4.4	12.4	15.5	15.5	16.5			7.35	7.35	7.31	7.18	7.16	7.09			45	56	69		71			
4	12.5	4	5	0.05	0.30	6.5	5.1	7.0	6.5				7.35	7.27	7.16	7.06	6.80				57	68	71		70			
5	11.6	5	12	0.05		1.0	1.5	8.8	10.6				7.22	7.19	7.24	7.16	7.11				46	62	66	69				
6	15.0	4			0.15	6.9	8.1	8.4	8.4				7.32	7.25	7.22	7.13					49	61	68	67				
7	20.4	5	76	7.2	0.18	2.1	4.5	11.7	12.1	14.1			7.37	7.32	7.18	7.16	7.14				50	62	65	70		77		
8	11.1	3 ½	9	0.03	0.12	5.5	10.0	13.1					7.37	7.34	7.14	7.03					50	58	66	60				
9	16.0	9 ½			0.09	5.1	9.3	8.9	9.3	7.5	7.5	5.5	7.45	7.23	7.20	7.18	7.17	7.13	7.18	7.07	43	66	70	74	75	75		
10	15.0	6 ¾			0.12	2.8	11.0	12.1	10.0	9.6	7.9		7.32	7.29	7.15	7.16	7.09	6.93	7.00		44	60	63	66	64	64	65	

[1] Total urine excreted following administration of iron.
[2] Total iron excreted in the urine following administration of iron.

Table II. Changes in blood volume during acute iron poisoning

Dog No.	Red cell mass (ml)								Plasma volume (ml)								Total blood volume (ml)							
	Hours after injection of iron																							
	0	1	2	3	4	5	6	8	0	1	2	3	4	5	6	8	0	1	2	3	4	5	6	8
1	625	736			731		690		945	610			507		418		1570	1346		1238				
2			677						735	467								1144						
3	405	424	518			402			625	505	340			235			1030	929	858			637		
4	482	484		302					450	360		190					932	848		492				
5	520	587	535	505	521				535	402	355	335	265				1050	989	890	840	786			
6	590	570	481						558	435	352						1148	1005	833					
	840	955	923	847	676				898	715	680	667	590				1738	1670	1603	1514	1266			
	335	377	380						495	377	290						830	754	670					
	651	745	605	637	667	570			747	662	457	510	617	445		421	1398	1397	1062	1147	1284	1015		
	630	711	673	673	581	587			640	460	415	382	333	322			1270	1170	1088	1055	914	809		

Table III. Hemodynamic changes in acute iron poisoning

Dog No.	Mean arterial BP (mm Hg)								Cardiac output (l/min)								Total peripheral			
	Hours after injection of iron																			
	0	1	2	3	4	5	6	8	0	1	2	3	4	5	6	8	0	1	2	3
1	125	100	105		100	65			1.6	0.7			0.4				4.7	8.0		
2	125	110	75	25					3.3	0.8							2.3	5.3		
3	165	160	145		145	110			3.4	1.7	0.9	0.7	0.6				2.5	5.6	9.5	11.6
4	125	125	105	100	50				1.5	0.7	0.3		0.08				5.0	10.7	19.7	
5	120	105	110	85	80				2.9	1.2	1.4		0.8				2.5	5.3	4.7	7.8
6	140	100	105	70					2.1	0.8	0.8	0.2					4.0	7.2	8.2	10.5
7	155	145	145	135	115				6.2	2.4	2.0	1.4					1.5	3.6	4.5	6.6
8	135	120	90	95					2.7	1.2	0.7	0.3					2.7	6.0	7.2	10.0
9	140	100	100	110	125	125	120	90	4.2	2.5	2.4	1.4	1.4	1.3	1.2	1.1	1.9	2.4	2.5	4.6
10	150	120	105	105	105	95	60		5.2	2.1	1.6	1.3	1.3	0.9	0.5		1.7	3.4	4.6	5.0

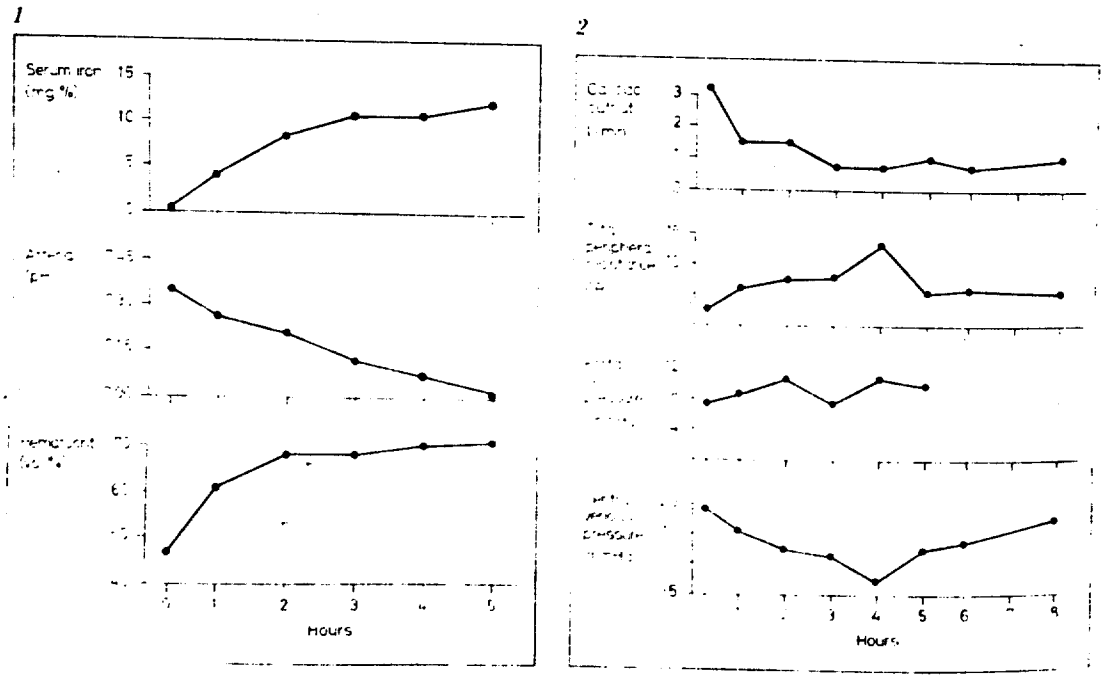
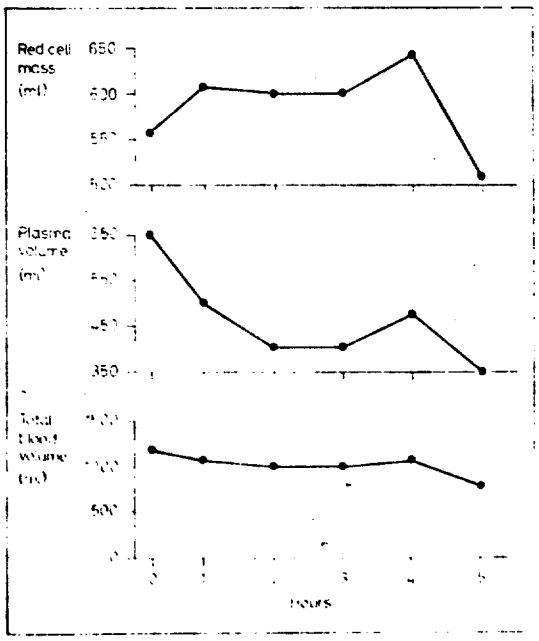


Fig 1-3 Mean ... during the course of iron poisoning.

resistance (PRU)				Portal vein pressure (mm Hg)								Central vein pressure (mm Hg)					
				Hours after injection of iron													
4	5	6	8	0	1	2	3	4	5	6	8	0	1	2	3	4	5
16.7				7.4	9.3			10.7	9.6	5.9		5.2	3.0			3.0	4.1
				8.1	8.9		5.9					4.4	3.3		4.4		
15.5				11.5	10.0	13.0	11.1		10.7			1.9	0.7	1.9			
37.5				8.1	10.7	12.6		10.7									
5.8				6.7	12.0	11.1	11.5	8.9				0.4	0.4	0.4	-1.1	-1.5	
				3.7	0.4	6.7	0.4					0.7	0.7	0.4	1.5		
													1.0	-1.1	-1.1		
												-3	-3	-3	-3.3		
5.1	6.0	6.0	4.9									1.8	1.8	1.8	1.5	-1.8	-1.5
4.8	4.8	6.1	6.1									1.1	0.7	0.4		-0.7	-1.1

3



Serial values for the ratio of the peripheral circulation hematocrit (obtained from independent measurement of the plasma volume and red cell mass) to the large vessel hematocrit (obtained from measurement of the hematocrit of venous blood) are presented in table V. Shifts occurred throughout the course of iron poisoning.

Figures 1, 2 and 3 depict hourly mean values of all parameters monitored in the study. The purpose of presenting the material in graphic form is to indicate trends. The graphs should not be used to ascertain the precise changes at given times because, in some instances, data are available on only a few animals (tables I, II and III).

Table IV. Alterations in circulation one hour after oral challenge (% change from normal)

Dog No.	Cardiac output	Mean arterial blood	Total peripheral resistance	Portal vein pressure	Central vein pressure	Red cell mass	Plasma volume	Total blood volume
1	−53	−20	+ 70	+26	−42	+18	−35	−14
2	−75	−40	+130	+10	−23		−36	
3	−57	− 3	+124	− 13	−63	+ 5	−19	−10
4	−54		+114	+32			−20	− 9
5	−59	−15	+112	+79		+13	−25	− 6
6	−61	−29	+ 80	+81		− 3	−22	−12
7	−58	− 3	+125			+ 3	−20	−10
8	−56	−11	+122			+13	−24	− 9
9	−60	−20	+100		−36	+13	−28	− 8
10	−41	−29	+ 26			+13	−12	
Range	−41 to −75	0 to −40	+ 26 to +130	+10 to +81	0 to −63	− 3 to +18	−12 to −36	0 to 14
Mean	−57	−17	+100	+36	−21	+ 8	−24	−9

Table V. Ratio of overall body hematocrit to venous hematocrit

Dog	Hours after iron poisoning						
No.	0	1	2	3	4	5	6
1	0.895	0.852		0.843			0.881
2			0.826				
3	0.871	0.816	0.869		0.893		
4	0.912	0.841		0.865			
5		0.956	0.909	0.870	1.027		
6		0.934	0.853				
7	0.972	0.921	0.880	0.800	0.720		
8	0.810	0.862	0.862				
9		0.915	0.886	0.932	0.851	0.967	
10		0.897	0.905	0.84	0.813	0.875	

Discussion

REISMANN and COLEMAN [7] presented data on the hemodynamic status of one dog with acute iron poisoning and stated that it was representative of data on three animals. They assumed that the early onset of acidosis in that dog was not related to tissue hypoxia because the cardiac output was not altered at two hours. In each of the 10 dogs we studied, however, we found marked reduction in cardiac output at one hour. Thus, tissue anoxia does provide a satisfactory explanation for the acidosis. Poor tissue perfusion leads to tissue anoxia, anaerobic metabolism, and an accumulation of lactic acid. Theoretically, decreases in cardiac output can be related to: (a) cardiac failure; (b) a decrease in total blood volume; or, (c) a decrease in effective circulating blood volume [1]. Congestive heart failure was probably not present because the central venous pressure was not elevated. There was a diminution in total blood volume, but the magnitude was too small to account for the degree of alteration in cardiac output. Thus, the early decrease in cardiac output appeared to reflect a reduction in the effective circulating blood volume, presumably from venous pooling.

Although there was no significant early change, there was a progressive reduction in total blood volume which contributed to the late decline in cardiac output. The magnitude of the reduction in blood volume in fatal iron poisoning has not been assessed previously. The only published data on changes of blood volume were obtained during the course of three nonlethal poisonings [7]. Several investigators have indicated

that blood volume studies in dogs are reliable only when red cell mass and plasma volume are measured simultaneously and independently [1, 2, 4]. GRABLE *et al.* [2] utilized this type of monitoring in a study of hemorrhagic and endotoxic shock and did not find a significant preterminal deficit in total blood volume (mean 7%). Through the use of this technique, the preterminal deficit in total blood volume in our 10 dogs with acute iron poisoning averaged 30%. The deficit was primarily the result of a decrease in plasma volume. The mechanism responsible for the reduction in plasma volume in hemorrhagic and endotoxic shock is assumed to be a loss of plasma due to increased intracapillary pressure (capillary stasis) [2]. The magnitude of the change in plasma volume found in dogs with iron poisoning was considerably greater than the loss associated with capillary stasis [2] and suggests the presence of increased capillary permeability unrelated to pressure changes. Elevated levels of vasoactive substances (serotonin and histamine) in plasma have been detected during acute experimental iron poisoning [11] and these substances could produce an increase in capillary permeability. This increase could also be the result of direct contact of iron with blood vessels which can cause cellular injury, for iron particles have been found in the lumen of intestinal vessels of iron poisoned animals [8, 9, 10].

An increase in the peripheral circulation hematocrit was observed in this study. As hemoconcentration occurs, viscosity is increased and resistance to flow elevated. To what extent these factors played a role in the reduction in cardiac output cannot be estimated because the dynamic relations between myocardial capacity, viscosity, peripheral resistance, and blood flow have not been established in intact animals.

Although shock is a cardinal feature of fatal acute iron poisoning in children, no hemodynamic data obtained on children during acute iron poisoning have been published. Whether the observations made on dogs would apply precisely to children is unknown. One important difference probably exists. In unsplenectomized dogs in shock or in impending shock, there is an influx of blood with a high hematocrit from the spleen. To some extent, the increment in large vessel hematocrit in dogs is the result of this factor. In humans, this mechanism is not operative to any significant degree. Thus, the reduction in total blood volume in children might be far greater than that found in dogs.

Until data on humans are available, therapy must be based upon information gained from animals studied under experimental conditions. This study suggests that the need to restore effective blood volume occurs early in the course of fatal iron poisoning and that the presence of this need cannot be predicted by measuring changes in arterial blood pressure.

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14. Requests for reprints should be addressed to: C.F. WHITTEN, M.D. General Clinical Research Center for Children, Children's Hospital of Michigan, 3224 St. Antoine, Detroit, Mich. 48202, USA.

PYLORIC STENOSIS COMPLICATING ACUTE POISONING BY FERROUS SULPHATE

MARY J. WELMERS
M.D. Lond., F.R.C.P.
PHYSICIAN TO THE CHILDREN'S DEPARTMENT

A. J. HERIOT
M.B., M.S. Lond., F.R.C.S.
SURGEON

KING'S COLLEGE HOSPITAL, LONDON

ACUTE iron poisoning, usually due to the ingestion of large numbers of 'Fersolate' tablets by very young children, is reported from time to time. Thomson (1947, 1950) has reported six cases, two of which were fatal, in children aged from 11 months to 4½ years, and Spencer (1951) a further eight cases, four of which were fatal, in children aged 12–23 months. Of the reported necropsy findings (Thomson 1947, Forbes 1947, Prain 1949, Spencer 1951) the most striking were ulceration and necrosis of the gastric mucosa, sometimes extending down to the muscle coat; in the case reported by Smith et al. (1950) the brunt of the damage was in the ileum, although stomach and jejunum were also involved.

Vomiting, usually with repeated small haematemeses, is a constant symptom in the first twenty-four hours following the ingestion of ferrous sulphate in toxic doses, and in one child, aged 21 months, daily vomiting continued for twenty-five days and then ceased (Spencer 1951). In two cases severe pyloric stenosis developed, necessitating operation (Crosskey 1952, Ross 1953); in one of them (Crosskey 1952), a child, aged 3 years, who swallowed 67 ferrous-sulphate tablets, vomiting only became severe a month after admission to hospital, and pyloroplasty was followed by complete recovery. In Ross's (1953) case, a boy, aged 17 months, took only 6/12 tablets of fersolate, and severe vomiting did not develop until after his discharge from hospital on the thirteenth day. Gastrostomy was done on the forty-fifth day, and a jejunostomy nine days later, but the child died on the fifty-ninth day.

In the two cases described below severe pyloric obstruction developed and a successful gastro-enterostomy was done on the thirty-sixth and thirty-fifth days after the ingestion of the fersolate tablets.

CASE-RECORDS

Case 1.—A girl, aged 21 months, swallowed at 8.30 A.M. on Nov. 7, 1944, an unknown number of fersolate tablets prescribed for her mother. At 9 A.M. she vomited and brought up eight tablets. She was admitted to another hospital at 3.15 P.M. On admission there she looked very ill and was restless, with a very weak and rapid pulse and some cyanosis of extremities. Shortly after admission she vomited blood. Her stomach was washed out at 6.30 P.M., and a feed of milk and egg was given. She continued to vomit bloodstained fluid next day. From then on she vomited daily, usually one large vomit. She lost weight rapidly, was very constipated and became somewhat dehydrated. On Dec. 5, four weeks after she had swallowed the fersolate tablets, a barium meal showed gross delay in emptying of the stomach, with a large residue after eight hours. She was transferred to King's College Hospital.

On admission on Dec. 6, 1944, she was very listless and looked ill, emaciated, and dehydrated. An ill-defined mass was palpated under the left costal margin, but the stomach did not seem to be dilated, and gastric peristalsis was not visible. A barium meal showed a filling defect at the fundus, contracture of the lesser curvature, and severe pyloric stenosis (fig. 1). Vomiting was copious, and even clear fluids were not retained.

Treatment.—An intravenous saline infusion was set up on the day after admission and continued for ten days (until Dec. 17). On Dec. 13 a laparotomy was done through a paramedian incision under general anæsthesia. The whole stomach wall was thickened and œdematous and had caused narrowing and obstruction of the pyloric canal. A posterior gastrojejunostomy through the mesocolon was done. All the coats of the stomach were inflamed and thickened.

Progress.—Vomiting ceased next day, and within four days full feeding was established. The child made an uninterrupted recovery and was discharged on Jan. 2, 1945, weighing 21 lb. 3 oz. She attended as an outpatient once and then stopped attending.

Follow-up.—On Nov. 17, 1953, at the age of 10 years 9 months, she was well grown and weighed 4 st. 11 lb. On abdominal palpation nothing abnormal was detected. A barium meal showed the gastro-enterostomy to be working well; no barium passed through the pylorus, and none could be forced through on screening. The filling defect, although much smaller, was still present at the fundus (fig. 2).

Case 2.—A boy, aged 2 years, was found at 4 P.M. on Oct. 5, 1953, chewing the last of 40 fersolate tablets. He was given a seidlitz powder, after which he vomited brown fluid, became very drowsy, and was brought at 4.35 P.M. to the casualty department, where his stomach was immediately washed out with 2½% sodium-bicarbonate solution. The stomach contents consisted of much brown fluid in which broken fersolate tablets were easily recognisable, and smelled strongly of ferrous sulphate. The child was in bed in the ward by 6 P.M. He was then extremely cold, pale, and shocked, with a weak rapid pulse and some cyanosis of extremities. He vomited small quantities of pure blood frequently and passed a soft black stool which smelled of iron.

Treatment and Progress.—A continuous intravenous saline infusion was put up at 8 P.M. During the evening the boy passed two more black stools and continued to vomit blood until the early hours of the morning. Bismuth carbonate gr. ½ four-hourly was given from the time of admission, but most of this was probably vomited. Next morning (Oct. 6) his general condition was greatly improved, and during the afternoon (twenty-four hours after taking the iron) he took sips of water by mouth. At least once during the day he had a "coffee grounds" vomit. Early on Oct. 7 he became jaundiced and comatose. His liver became enlarged during the day, the coma deepened, and he had convulsions and became oedem-

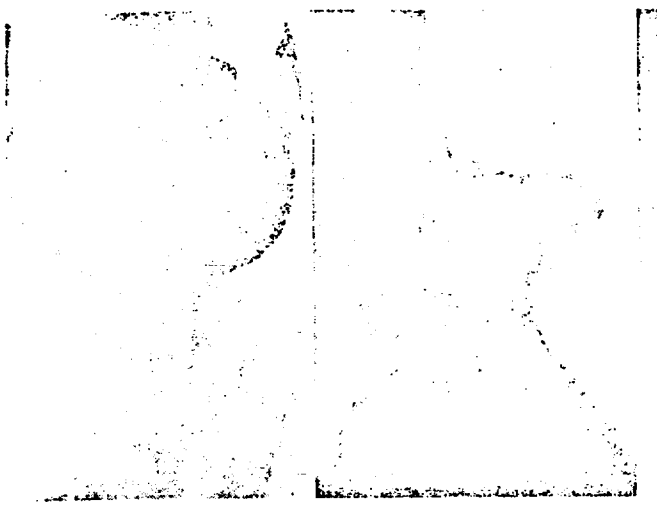

Fig. 1

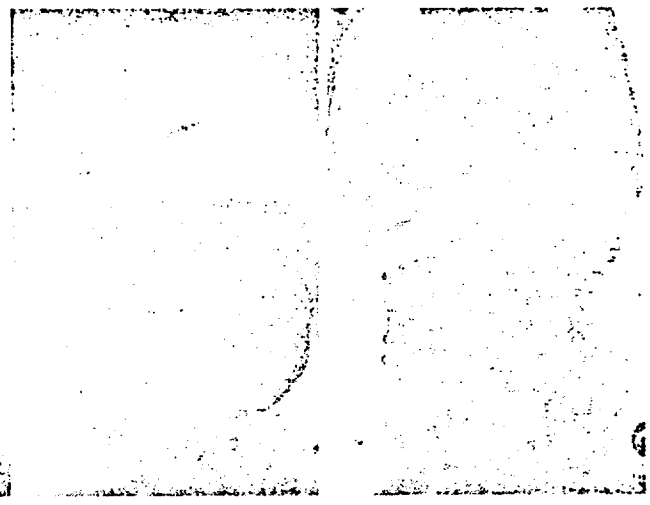

Fig. 2

Fig. 1—Delayed emptying of stomach and filling defect at fundus (case 1 on admission).

Fig. 2—Gastro-enterostomy working and no barium passing through pylorus (case 1 nine years later).

Fig. 3

Fig. 4

Fig. 3—Narrowing of pylorus and contracture of lesser curvature of stomach after barium meal in case 2.

Fig. 4—Large gastric residue twenty-four hours after barium meal in case 2.

tous. At midday on Oct. 7 (forty-four hours after taking the tablets) 30 oz. of bloodstained fluid was vomited. Owing to his critical general condition nothing was given by mouth until Oct. 9, when small quantities of water were allowed. On Oct. 10 he took 8 oz. of clear fluid by mouth and vomited twice; one vomit contained black shreds, which were thought to be iron-stained gastric mucosa. On Oct. 11, 15 oz. of clear fluids was taken without vomiting. The stools were still black, and the first normal-coloured stool was passed on Oct. 12 (a week after ingestion of tablets). By Oct. 13 he was taking milk and milk puddings, and by Oct. 15 a soft light diet. That day he vomited once. By Oct. 18 he was enjoying his food but vomiting small quantities immediately after meals; this vomiting increased steadily during the following days. He seemed hungry, ate a good diet eagerly, and cried if the food-trolley passed him by. For some days it was not realised by the nurses how much food he was losing in the vomit, but he became extremely constipated and began to look wasted. On Oct. 27 he weighed 20 lb. 8 oz. A barium meal on Oct. 28 showed a stricture of the pylorus and some fibrous contracture of the lesser curve of the stomach (fig. 3). There was a large gastric residue after twenty-four hours (fig. 4). It was decided to do a gastro-enterostomy, but the operation was postponed because the child's general condition was still so poor. An attempt was made to improve his nutrition by feeding him on lactic-acid milk alone, on the assumption that the fine curds would pass through the narrowed pylorus, and after a few days beaten-up raw egg and sugar and, later, a high-protein diet liquefied in a 'Turmix' homogeniser were added. However, vomiting about twice a day continued, there was no gain in weight, and laparotomy was done on Nov. 17, 1953. On the day of operation the boy weighed 20 lb. The findings at operation were similar to those in case 1, except that the inflammation involved only the pylorus. Again a posterior gastrojejunostomy was done. The liver appeared normal.

Postoperatively the boy made an excellent recovery and was discharged on Nov. 1 weighing 22 lb. 12 oz.

COMMENTS

At the time of our first case we were not aware of any toxic effects attributable to large doses of ferrous sulphate and so did not connect either the symptoms of pyloric stenosis or the appearance of the stomach at operation with the ingestion, four weeks previously, of an indefinite number of ferrous-sulphate tablets. It was not until Forbes (1947) and Thomson (1947) reported their cases that we correctly diagnosed case 1 retrospectively.

The severe gastritis found at all the necropsies can be produced in laboratory animals by feeding toxic doses of ferrous sulphate (Forbes 1917).

Spencer (1951) points out that in ferrous-sulphate poisoning there are two main danger periods: in the first four to six hours death may result from severe shock caused by corrosion of the stomach; and after a latent period of twelve to twenty-four hours absorbed iron may damage the liver so severely as to cause hepatic failure. We draw attention to a third danger, which is greatly delayed, much less acute, and more amenable to treatment. In the twenty-two cases of ferrous-sulphate poisoning hitherto reported in English journals (including our own two cases) nine patients died in the acute stage, and five developed pyloric stenosis of whom one died. From the two cases of Crosskey (1952) and Ross (1953) and the two present cases it seems to take about four weeks for the full clinical picture of pyloric obstruction to develop. One of Spencer's (1951) patients was discharged on the eleventh day, but two weeks later seemed very unwell and had vomited at least once a day at home. However, after a further four weeks he was eating well and not vomiting. This child may have had less severe pyloric obstruction which was spontaneously relieved as the inflammation gradually subsided.

With general recognition of the dangers of ferrous-sulphate poisoning and more effective treatment of the acute stages one may expect an increase in the incidence of pyloric stenosis as a late complication.

SUMMARY

Two cases of pyloric stenosis following the ingestion of large numbers of fersolate tablets are described.

Both were successfully treated by gastro-enterostomy.

ADDENDUM

A further case of pyloric stenosis, in a child of 16 months, treated by partial gastrectomy, has been published by Elliot-Smith and Davies (1954). The distal half of the stomach, which was thickened, rigid, and fibrotic, was removed, and the child made a good recovery.

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Acute Ferrous Sulfate Poisoning

A Histochemical Study of Its Effect on the Liver

C. L. WITZLEBEN, MD, SAN FRANCISCO, AND N. J. CHAFFEY, BSc, OAKLAND, CALIF

The hepatic lesions induced by acute ferrous sulfate overload have been studied by enzyme histochemistry. Changes in hepatocellular enzyme activity appeared within eight hours after ferrous sulfate injection. The initial enzyme changes consisted of an apparent increase in activity of a number of oxidative enzymes and glucose-6-phosphatase (G-6-P) in the parenchymal cells. This was followed by a loss of activity of these enzymes in the same areas which previously showed the increase. The observed disturbances in hepatocellular oxidative enzyme activities suggest a probable biochemical basis for the toxicity of acute ferrous sulfate overload in animals and man.

ALTHOUGH acute iron overdosage is one of the most common causes of fatal poisoning in children in the United States, little is known of its biochemical and morphologic effects.

Two experimental studies have been carried out[1,2] demonstrating that acute hepatic injury can be induced by ferrous sulfate intoxication, but these studies relied only on standard histologic stains in the examination of the liver and provided no information about the chemical effects of this intoxication on the liver cells. The studies reported here were done to elucidate some of these by the use of enzyme histochemistry.

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From the Department of Pathology, University of California, San Francisco, and Children's Hospital Medical Center of Northern California, Oakland, Calif. Dr. Witzleben is currently at St. Louis University Medical School; Mr. Chaffey at the Cardinal Glennon Memorial Hospital for Children, 1465 S Grand Blvd, St. Louis, Mo.

Reprint requests to 1402 S Grand, St. Louis 63116 (Dr. Witzleben).

Materials and Methods

Forty New Zealand white female 2 kg rabbits were used, and iron was injected intravenously in test animals as a 25% aqueous solution of $FeSO_4 \cdot 7H_2O$. With the administration of a single dose in the amount of 90 mg/kg, the incidence of hepatic necrosis was variable, and it was found that two additional injections of 50 and 90 mg at one and five hours respectively after the first injection, resulted in a higher incidence of hepatocellular injury in the particular strain of rabbits used. Test and control animals were fasted from the time of the initial injection, but were given water ad libitum. Control animals were not injected. Four animals were sacrificed at 4 hours after the initial injection, and 10 each at 8 and 12 hours after the initial injections. Blocks of livers were immediately frozen with solidified carbon dioxide. Additional slices from the same areas were fixed in formalin. The formalin-fixed material was embedded in paraffin, and sections were cut and stained with hematoxylin and eosin, and Perl's stain. Sections of frozen tissue were cut with a cryostat and stained with oil red O for neutral fat, PAS for glycogen, for succinic dehydrogenase (SDH),[3] reduced triphosphopyridine nucleotide diaphorase,[4] reduced diphosphopyridine nucleotide diaphorase (DPND),[4] cytochrome oxidase,[5] glucose-6-phosphatase (G-6-P),[6] glutamic dehydrogenase,[7] and adenosine triphosphatase (ATPase).[8]

Results

Immediately following the injection of ferrous sulfate, many animals had convulsions. The subsequent development of lesions did not show any apparent correlation with convulsions. Frequently the convulsions were followed by a brief period of apnea. Occasionally animals did not recover and died at this time. Such severe reactions were much less common after the succeeding injections. Animals surviving the initial injection usually showed no abnormality for the next several hours, other than hyperpnea. By

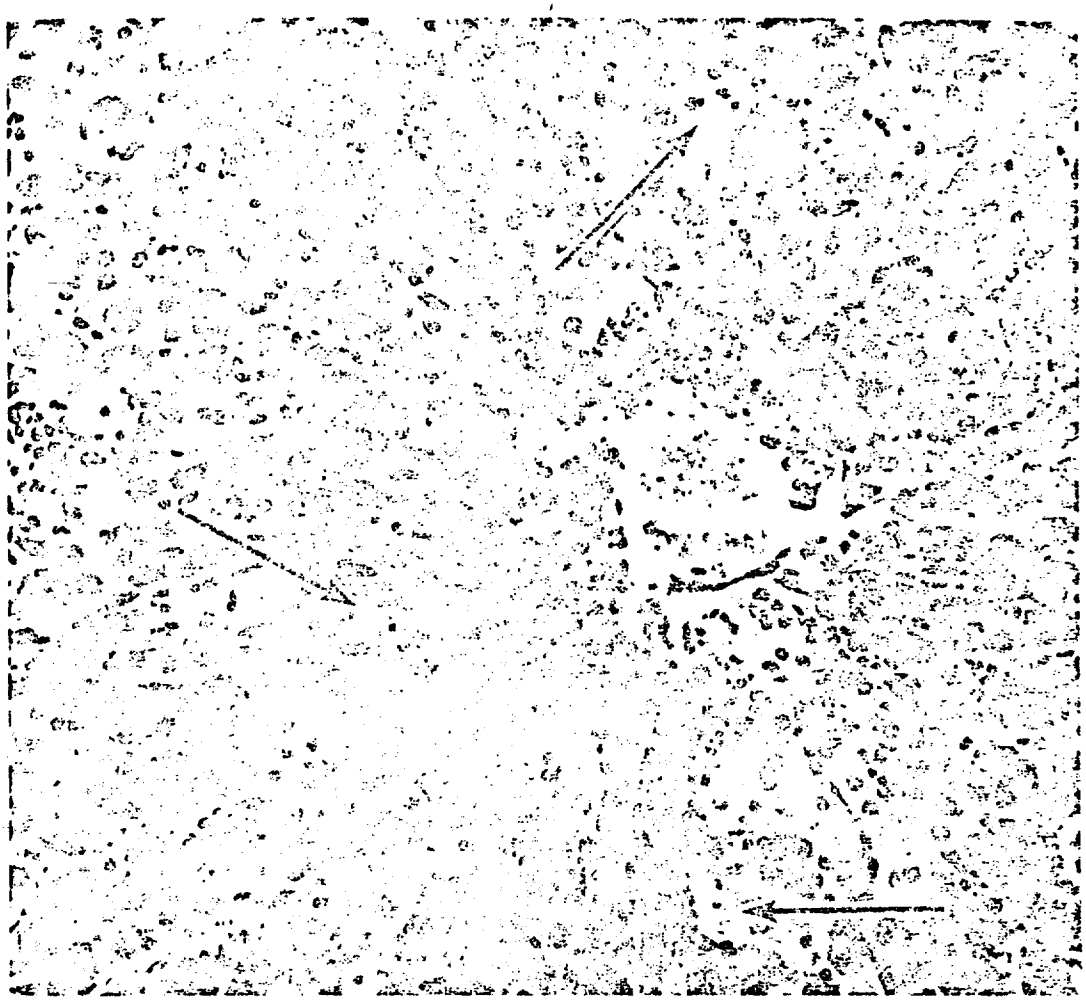

Fig 1.—Liver, eight hours after initial $FeSO_4$ injection. There is a diffuse severe loss of basophilia, many pale hyalinized prenecrotic cells are seen, and a number of frankly necrotic cells are present, several of which are indicated (*arrows*) (hematoxylin and eosin, × 100).

ginning about six hours after the initial injection, a few animals showed increasing weakness and lethargy, and finally became moribund. Others showed no apparent ill effects, other than persistent hyperpnea, until they were killed.

Histologic Findings.—At four hours, there was in most animals an evident loss of stainable glycogen from the periportal parenchymal cells, as compared controls. A slight increase in stainable fat was present in test animals. There was a massive increase in stainable ferric iron in the littoral cells in all test animals and hemosiderin granules were seen in a few periportal parenchymal cells. The plasma in occasional portal veins stained diffusely blue with Perl's reaction. Necrotic periportal cells were seen, but these were quite uncommon.

Eight hours after the initial injection, more significant alterations of the parenchymal cells were present in most of the animals. These alterations varied in severity from a slight loss of cytoplasmic basophilia through marked loss of basophilia with hyalinization of the cytoplasm (which we interpret as a "prenecrotic" change) (Fig 1), to frank necrosis. Polymorphonuclear leukocytes were often found in relation to necrotic cells. These parenchymal alterations tended to begin in the periportal cells and extend centrally. Increased neutral fat was found in parenchymal cells, especially those in the periportal region. Stainable iron was further increased in the littoral cells at this time and hemosiderin granules were also seen in larger numbers of periportal parenchymal cells than previously. A few periportal parenchymal

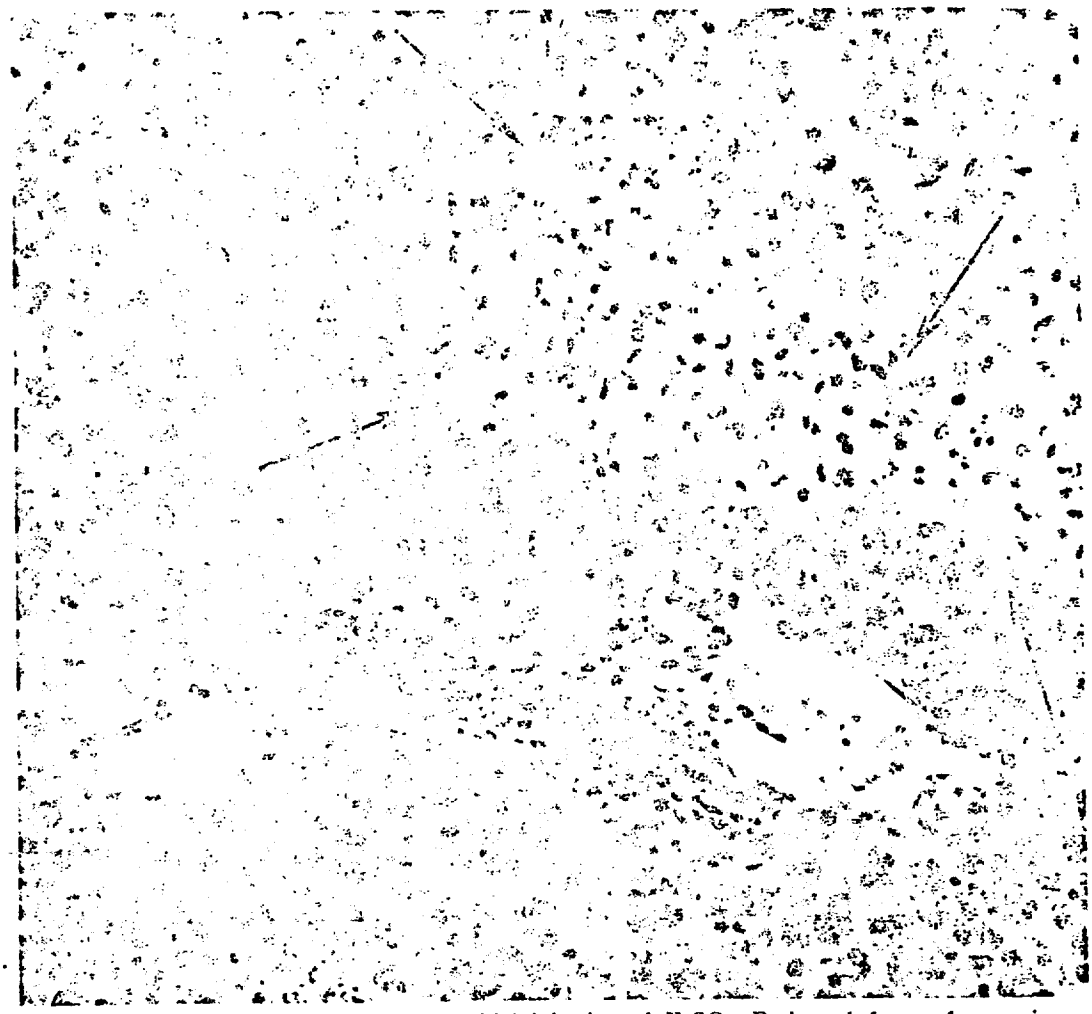

Fig 2.—Liver, eight hours after initial injection of $FeSO_4$. Periportal focus of necrosis outlined (*arrows*) (hematoxylin and eosin, × 100).

cells showed a diffuse blue-staining of the cytoplasm. Some of these diffusely blue cells were obviously necrotic, but conversely, many necrotic cells showed no stainable iron. The necrotic and prenecrotic changes varied considerably in extent from animal to animal, and were either generalized (Fig 1), involved clusters of cells (Fig 2), or scattered necrotic single cells separated by normal cells (Fig 3). They were, however, quite consistent throughout the liver of each individual animal. Because of the variation from animal to animal, it was possible to reconstruct all degrees of injury, from minimal loss of basophilia to widespread frank necrosis, by studying animals killed at 8 and 12 hours.

At 12 hours, essentially similar but somewhat more widespread changes were found. The lesions induced, as studied by these stains, appeared to be essentially similar to those described previously,[1,2] but they developed more rapidly.

Enzyme Histochemistry.—At four hours, no consistent changes in enzyme activity were found. Enzyme changes were found in animals killed at eight hours whose livers showed at least scattered necrotic cells and considerable loss of basophilia. In animals which did show these changes, the first enzyme alteration was noted. This alteration consisted of an irregular increase in periportal enzyme activity (Fig 4, *bottom* and Fig 5). In some livers, this increase tended to be more widespread, or at least more readily recognized, than either the evident necrosis or the prenecrotic hyalinization, even though it did not appear until

these were in evidence. All oxidative enzymes studied appeared to be affected to the same extent except for cytochrome oxidase which was somewhat less markedly altered. G-6-P activity was quite markedly increased, and, as demonstrated by serial sections, often in the same cells which showed the increase in activity of the oxidative enzymes. ATPase activity, however, showed no appreciable alteration. The described increase did not appear to be due to the presence of lipid in these cells,[9] since it was not eliminated by rinsing of the sections in acetone.

The most severe enzyme alterations were seen at 8 and 12 hours in the livers which showed the most marked evidences of injury with staining by hematoxylin and eosin (marked loss of basophilia, extensive prenecrotic changes, and extensive necrosis) and consisted of a marked loss of enzyme activity from parenchymal cells (Fig 6). Cells adjacent to or within these areas of loss in activity often stained with increased intensity. The changes involved all the oxidative enzymes examined and G-6-P as well (Fig 7). There was no striking difference in the extent of alteration of any of the oxidative enzymes or G-6-P, although it appeared that cytochrome oxidase was somewhat less affected than the other oxidative enzymes examined. ATPase was less affected and often showed little alteration except for a tendency for central increase. The enzyme changes closely matched in distribution the alterations seen in paraffin sections and were focal (Fig 6) or were generalized (Fig 8 and 9) in conformity with the distribution of lesions as visualized with staining by hematoxylin and eosin.

Comment

The changes in hepatic enzyme activity which developed in acute iron overload were closely related in time and location to abnormalities demonstrable with staining by hematoxylin and eosin and were not seen until these were evident. The changes seen with staining by hematoxylin and eosin were similar in location to those previously described in acute iron overload.[1,2]

In livers damaged by murine hepatitis virus,[10] the observation also has been made that focal enzyme abnormalities did not develop until focal changes were visible with hematoxylin and eosin. In the hepatitis studies, however, generalized enzyme alterations were discovered before changes

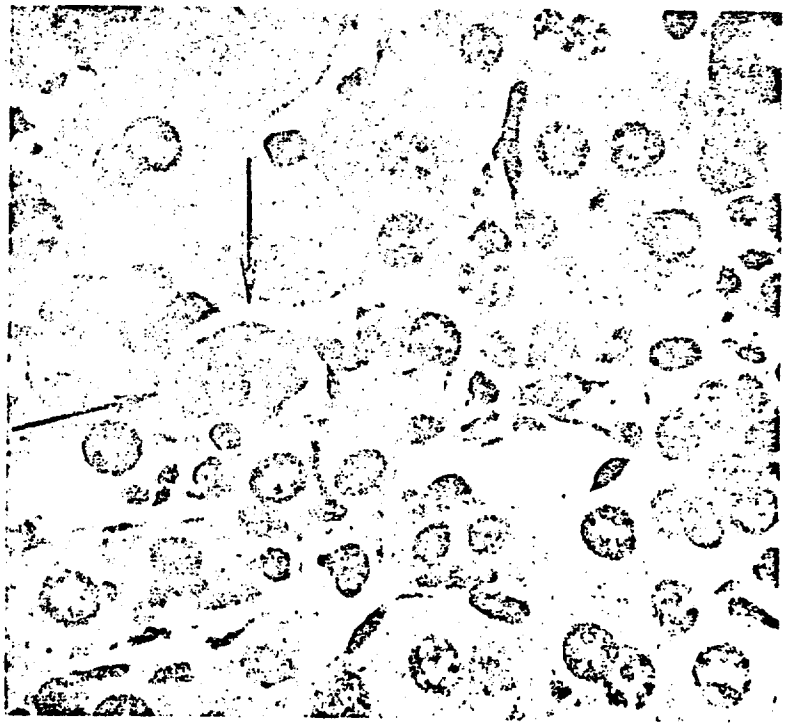

Fig 3.—Liver, eight hours after initial ferrous sulfate injection. Single necrotic cell (*arrows*) with nuclear karyorrhexis. (hematoxylin and eosin, × 450).

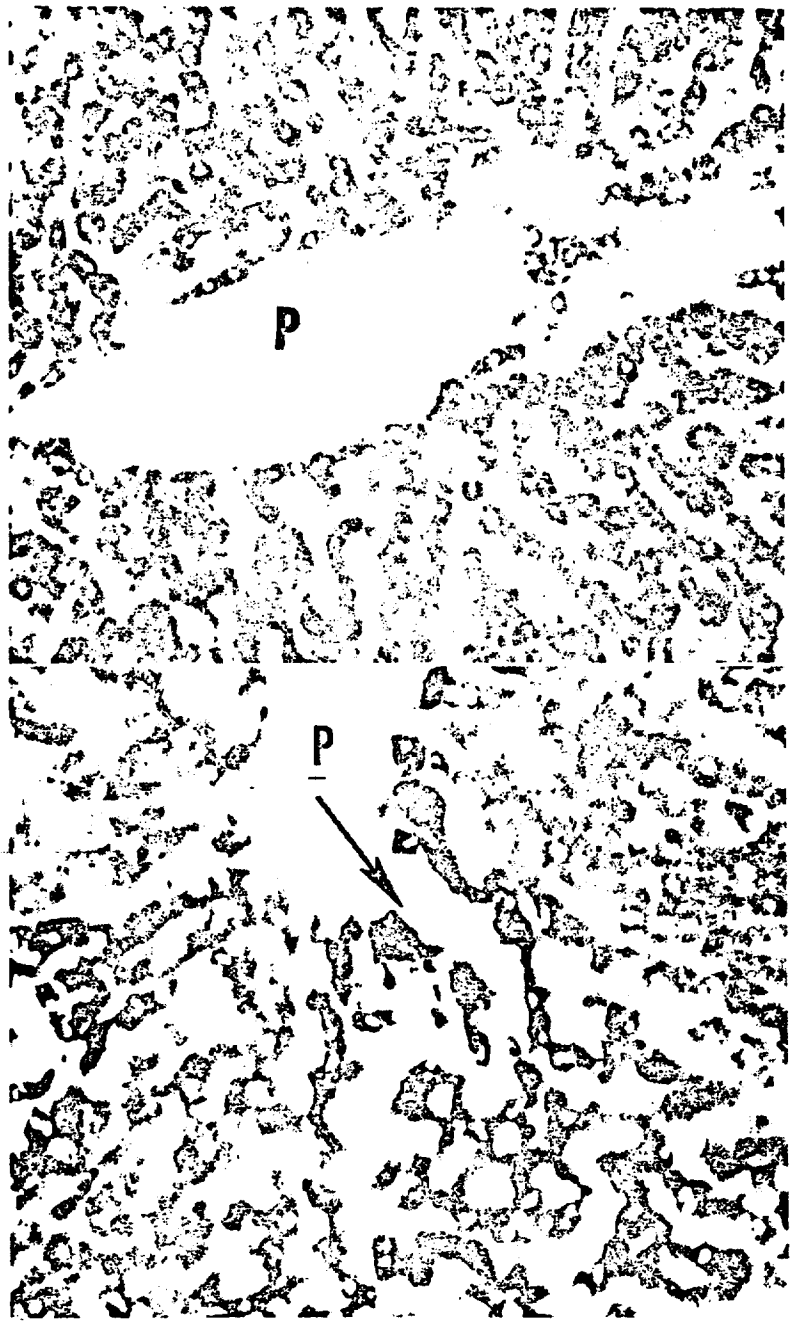

Fig 4.—*Top*, Control liver, stained for glucose-6-phosphatase activity. Cells surrounding portal tract (*P*) stain relatively uniformly (G-6-P, × 100). *Bottom*, Portal tract (*P*), with several adjacent foci of increased staining of parenchymal cells. Largest focus is indicated (*arrow*). In this liver, frankly necrotic areas of this size were not present eight hours after ferrous sulfate injection (G-6-P, × 100).

were visible with hematoxylin and eosin. These generalized changes were not seen, however, until 40 hours after the initial injection of virus whereas animals in our study were killed within 12 hours of the initial injection. The "superimposition" of lesions demonstrable with hematoxylin and eosin and enzyme histochemical changes in this experiment was probably due to (1) the rapidity of development of the lesions, and (2) the relative insensitivity of enzyme histochemical techniques in detecting cellular injury. In terms of sensitivity of these techniques, it is to be noted, however, that the early enzyme histochemical changes were occasionally apparently more widespread or at least most readily recognizable than lesions demonstrable with hematoxylin and eosin.

The initial recognizable enzyme alteration was an increase in intensity of staining in the periportal parenchyma. This was closely

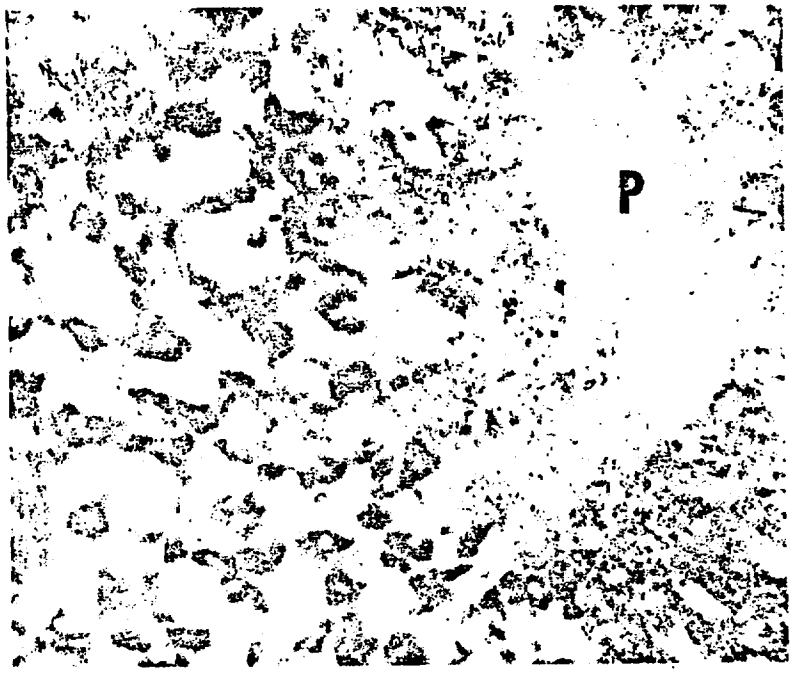

Fig 5.—Portal tract (*P*), with increased succinic dehydrogenase staining in periportal focus eight hours after ferrous sulfate injection (SDH, × 120).

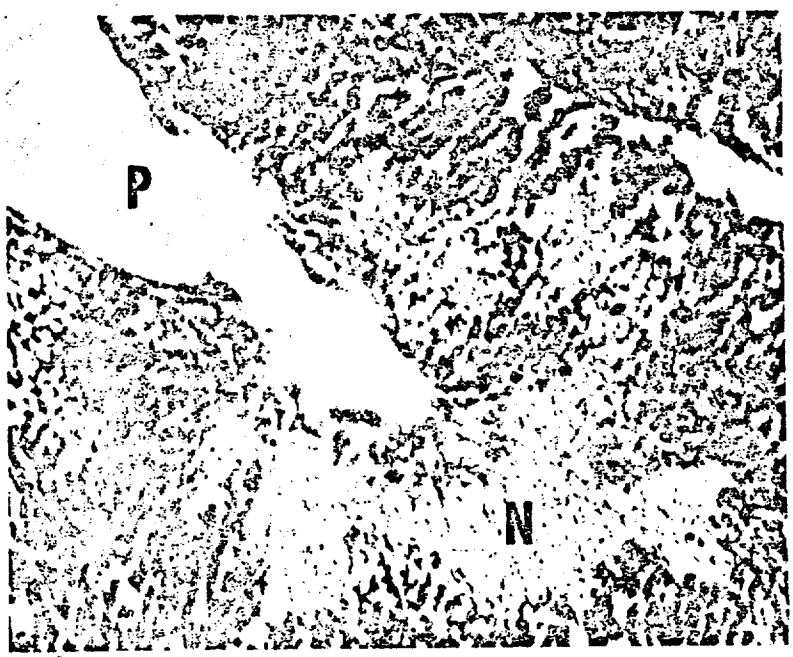

Fig 6.—Portal tract (*P*), with adjacent necrotic focus (*N*) showing marked loss of enzyme activity eight hours after ferrous sulfate injection (DPND, × 50).

followed by a loss of staining in developing focal or generalized areas of necrosis. An apparent increase in enzyme reaction product has been described previously in experimental liver injury.[9] A probable explanation for this seeming increase is that induced disturbances in the cellular membranes and/or organelles makes more enzyme available and increases the reaction rate, with a resulting increase in stainable product.[11] The subsequent loss of demonstrable activity is presumably due to an increase in the severity of injury, with either a resultant leakage of enzyme from the cells or actual destruction of enzyme.

The difficulties inherent in the biochemical interpretation of enzyme histochemical results are well known, but the results of this experiment appear at least to demonstrate that acute massive iron overload is capable

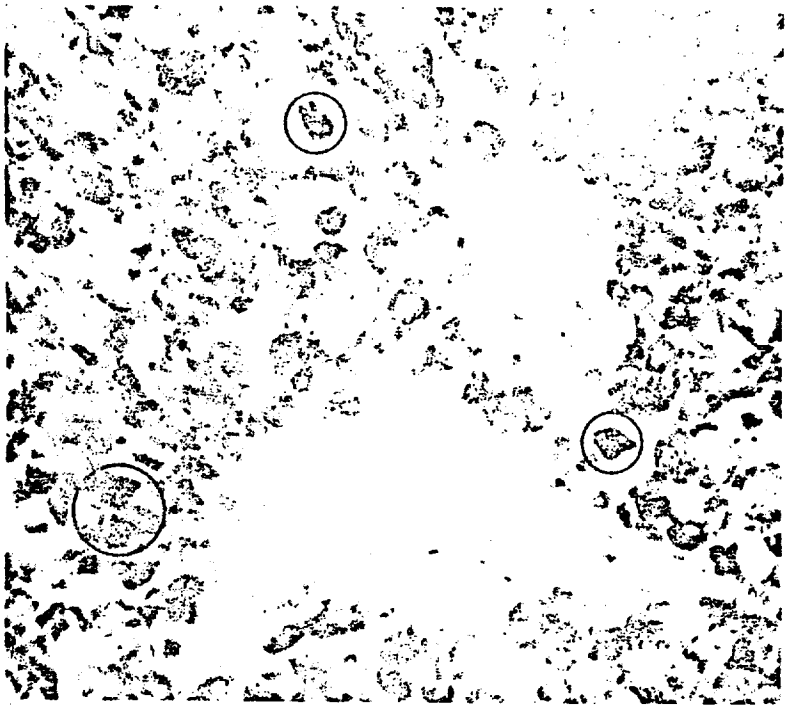

Fig 7.—G-6-P activity eight hours after initial $FeSO_4$ injection. Many cells have lost staining activity, while others (some are circled) show increased staining (G-6-P, × 100).

Fig 8.—Control liver. DPND activity. The regular pattern with centrilobular accentuation is evident (DPND, × 30).

of causing alterations in a number of cellular oxidative enzymes, including several in the Krebs cycle. The citric and lactic acidemia seen in acute ferrous sulfate overload[12] would result from the damage to Krebs cycle enzymes here demonstrated histochemically. The occurrence of similar biochemical lesions involving more critical organs would also serve as a biochemical basis for the frequently unexplained death in acute ferrous sulfate intoxication in man.[13]

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Generic and Trade Names of Drug

Ferrous sulfate—*Feosol, Ferro-Theron, Irosul.*

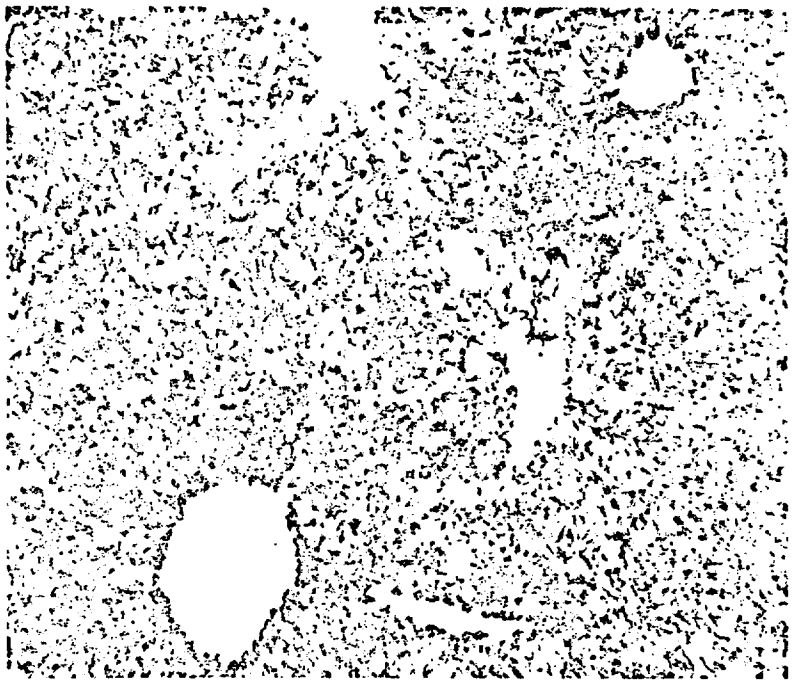

Fig 9.—DPND activity in liver eight hours after initial $FeSO_4$ injection, showing generalized alteration in enzyme activity with loss of regular pattern due to irregular loss and gain of activity. Same liver in Fig 1, with diffuse abnormalities on staining by hematoxylin and eosin. Similar changes are shown in Fig 7 (DPND, × 30).

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